Proceedings of the

XII Latin-American Congress of Artificial Organs and Biomaterials

"Integrating and strengthening the Latin-American Biomaterials' Community

12-15 de diciembre 2023 │ Mar del Plata │ Argentina

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and

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Preface

The **XII Latin-American Congress of Artificial Organs and Biomaterials, COLAOB 2023,** was held in the charming city of Mar del Plata, Argentina, from December 12th to 15th, 2023. This biennial event was a collaborative effort between the Research Institute for Materials Science and Technology (INTEMA, UNMdP-CONICET) and the Laboratory of Osteo-articular Biology, Tissue Engineering and Emerging Therapies Research, (LABOATEM, UNR-CONICET) in Argentina.

COLAOB's primary objective is to convene scientists from the realms of academia and industry who share a profound interest in the domains of biomaterials science and engineering, artificial organs, and tissue engineering. Furthermore, the congress serves as a platform to deliberate on cutting-edge developments in these fields.

Under the guiding motto of the congress, “Integrating and Strengthening the Latin-American Biomaterials Community” we aspire to consolidate institutional affiliations and promote scientific discourse among Latin-American research institutions, universities, and companies engaged in the development and marketing of biomedical products.

Since the inception of COLAOB up to the present, this congress series has consistently provided a unique opportunity for researchers, professionals, and PhD students to interact with thought leaders in their respective fields and present their achievements through both oral and poster presentations.

On behalf of the Organizing Committee, we are honored to present this **Proceedings book** that contains the peer-reviewed abstracts of oral and poster presentations.

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Joaquim **Vives** (Blood and Tissue Bank, Spain)

Topics

T1. Additive manufacturing (including Biofabrication)

T2. Biomaterials characterization

T3. Biomaterials for tissue engineering and regenerative medicine

T4. Cell-biomaterial interactions

T5. Drug release systems

T6. Functionalization of biomaterials

T7. Key enabling technologies (including lab-on-a-chip, artificial intelligence)

T8. Legal, ethical and regulatory aspects

T9. Medical implants and medical devices

T10. Modeling and simulations

T11. Nanobiomaterials

T12. Tissue and organ models

Invited Plenary Speakers

Anthony **Atala** (Wake Forest Institute for Regenerative Medicine, NC, USA).
"Regenerative medicine: Current concepts and changing trends"

Daniel **Cohn** (Casali Center of Applied Chemistry, Institute of Chemistry, Hebrew University of Jerusalem, Israel).
"Designing multifunctional 3D printed medical devices"

Kattesh V. **Katti** (Institute of Green Nanotechnology, and University of Missouri Cancer Nanotechnology Platform, Missouri, USA).
"Next generation of nanobiomaterials are "Nano-ayurvedic medicine" Drugs - Immunomodulatory holistic medicine approach for cancer therapy - Through green nanotechnology"

Diego **Mantovani** (Département de génie des mines, de la métallurgie et des matériaux, Faculté des sciences et de génie, Université Laval, Québec, Canada).
"Nanocoatings, degradable metals and in vitro models for the next generation of biomaterials for reparative and regenerative medicine"

Yannis **Missirlis** (University of Patras, Greece)
"Controversies and key nano insights in long standing physiological and biological concepts"

Lorenzo **Moroni** (MERLN Institute for Technology-Inspired Regenerative Medicine, Maastrich, The Netherlands).
"Form and function in tissue regeneration: which role can biofabrication play?"

Sergio **Moya** (Soft Matter Nanotechnology Lab, CIC biomaGUNE, San Sebastian, Spain)
"Biological Fate of Nanomaterials in Nanotoxicity and Nanomedicine research"

Alejandro **Sosnik** (Laboratory of Pharmaceutical Nanomaterials Science, Faculty of Materials Science and Engineering, Technion-Israel Institute of Technology).
"Sonodynamic therapy of cancer: Challenges and opportunities"

Keynote speakers

Paulo H. **de Souza Picciani** (IMA UFRJ, Brazil)
"Polymer-based nanostructures for tissue engineering"

Sara **Feldman** (Laboratorio de Biología Osteo-articular, Ing. Tisular y Terapias Emergentes LABOATEM, Fac. de Ciencias Médicas, Universidad Nacional de Rosario, Argentina).
"Recombinant proteins for bone regeneration"

Ademar B. **Lugão** (Laboratory of Polymeric Biomaterials and Nanoparticles for Theragnostic, Institute for Energy and Nuclear Research, IPEN, Brazil).
"New trends in advanced dressing"

Ângela Maria **Moraes** (School of Chemical Engineering University of Campinas, Brazil)
"Teaming up polysaccharide-based biomaterials and stem cells: joining attributes for enhanced lesion repair and tissue construction"

Ketul C. **Popat** (Department of Mechanical Engineering, School of Biomedical Engineering, Colorado State University, USA)
"Biomimetic micro/nano-engineering of material surfaces for cardiovascular implants"

Ana Paula **Rosifini Alves** (UNESP, São Paulo, Brazil)
"Biomaterials: sustainability and challenges"

ROUND TABLE

"What new challenges is tissue engineering facing?"

Coordinator

Sara Felman
LABOATEM, National University of Rosario, Argentina.

Expositors

Ângela Maria Moraes
School of Chemical Engineering University of Campinas, Brazil

Addressing Technical Challenges in Stem Cell Culture to Improve the Accessibility of Regenerative Medicine

The cultivation of stem cells may result in multiple cell types relevant to regenerative medicine applications. These cells may be successfully combined with biomaterials of different types and geometries and also with specific signaling molecules, increasing their applicability in regenerative medicine. However, the effective translation of the use of stem cells to the clinic is limited by several technical issues. For example, two-dimensional methodologies, often employed for cell culture in bench-scale research and initial *in vivo* and clinical studies, may not be sufficient to provide the required number of cells for larger scale application, becoming technically and economically unfeasible for more advanced clinical trials and product commercialization. Some of the issues related to cell culture on a larger scale will be discussed, with a view to increasing patients' access to regenerative medicine approaches involving cellularized biomaterials.

Alicia S. Lorenti
Jefe Terapias de Avanzada, Laboratorio Pablo Cassará, Buenos Aires, Argentina

Challenges in Regenerative Medicine / Tissue Engineering (RM/TE)

The objectives of new MR/IT technologies are fundamentally based on cells and biomaterials, and they constitute the focus of numerous research efforts. However, when the research goal is the translation of a product containing cells and biomaterials into clinical application (from "bench to bedside"), it is essential to identify and consider key aspects that go beyond in vitro developments. These aspects include technical issues (related to the acquisition, manipulation, and biosafety in the use of cells and tissues, as well as the acquisition, toxicity, and compatibility of the materials under consideration), ethical considerations (related to the origin and use of cells, the necessity of informed consent, among others), and regulatory matters (specific to each country, defining everything from the conditions of the workspace to the product's quality attributes).
These topics may be less "appealing" but are highly significant in ensuring a genuine commitment to the safety and efficacy of products for the benefit of patients.

Paulo Henrique de Souza Picciani
Instituto de Macromoléculas Professora Eloisa Mano – Universidade Federal do Rio de Janeiro (IMA/IFRJ), Brazil

When nanotechnology meets Tissue Engineering: potentialities and perspectives

Nanotechnology is currently a multidisciplinary and interdisciplinary branch of science that can produce and manipulate a variety of materials down to the nanoscale, i.e. below 100 nm. Nanomaterials are applied for a diversity of purposes and many of them have no related properties compared the bulk, macroscopic, materials. In the field of tissue engendering, nanomaterials can be applied to cell adhesion, morphology, viability, genetic regulation, apoptosis, and motility. New nanofabrication techniques can also control the local cellular microenvironment, produce nano-to-micro interconnectivity, and deliver drugs or bioactive agents to control cellular behavior. However, many relevant questions can be raised on the use of nanomaterials for tissue engineering, including scale-up possibilities, cost-effective processes regulatory issues, and environmental impacts. In this presentation, some of these relevant questions will be presented and discussed.

Joaquim Vives
Blood and Tissue Bank, Musculoskeletal Tissue Engineering Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain.

Towards a cell and tissue engineering platform to ensure rapid and sustainable patient access to innovative treatments

Blood, tissue and cell establishments (BTCs) stand out in the management of donor selection, procurement and processing of all types of substances of human origin (SoHO). In the last decades, the framework created around BTCs, including hospitals and national health system networks, and their links to research, development and innovation organizations and agencies have spurred their involvement in the study of groundbreaking advanced therapy medicinal products (ATMP), including Tissue Engineering Products. To further improve strategic synergies in the development of ATMPs, it will be required to create an international network involving BTCs and major stakeholders (i.e., research organizations, hospitals, universities, patient associations, public agencies). We will present our ethically responsible management model based on the values and missions of BTCs and their commitment to health equity, patient access and education (based on voluntary donation of SoHO to address unmet clinical needs, while contributing to training professionals).

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Oral presentations

O-01	Ângela Maria Moraes	COMPOSITE FILMS OF GELLAN GUM, FIBROIN, CARBOXYMETHYL CHITOSAN AND BIOGLASS AS POTENTIAL SKIN WOUND DRESSINGS
O-02	Katherina Fernandez	GRAPHENE OXIDE REDUCTION BY DOPAMINE
O-03	Cássia Priscila Cunha da Cruz	DEVELOPMENT OF RADIATION-INDUCED ALBUMIN-BASED NANOPARTICLES
O-04	Maria Lorena Gomez	ANTIMICROBIAL ANTIBIOTIC-FREE DRESSINGS FOR WOUND HEALING OBTAINED BY PHOTOPOLYMERIZATION
O-05	Adriana de Souza Rodrigues	SILVER NANOPARTICLES REDUCED BY TANNIC ACID AND SODIUM CITRATE: A SYNERGIC APPROACH WITH ANTIMICROBIAL PROPERTIES
O-06	Rachel Magnago	NANOCAPSULES BASED ON POLYCAPROLACTONE-TRIOL-BASED POLYURETHANE FOR A DRUG DELIVERY SYSTEM
O-07	Emilio Satoshi Hara	CELL MEMBRANE AS A POTENTIAL CELL-FREE THERAPEUTIC FOR RAPID BONE TISSUE ENGINEERING
O-08	Melina Hankovits	MAGNESIUM ALLOY (AZ91) FUNCTIONALIZATION WITH SILICA-GENTAMICIN NANOPARTICLES FOR BIODEGRADABLE IMPLANTS
O-09	Sara Feldman	OSTEOGENIC EFFECT OF COMPOSITE NANOFIBROUS SCAFFOLDS WITH OSTEOSTATIN
O-10	Paula A. Zapata	FABRICATION AND ASSESSMENT OF FUNCTIONAL POLYCAPROLACTONE/STARCH/CAO SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATION
O-11	Taynah Pereira Galdino	CHITOSAN/JATROPHA MOLLISSIMA-BASED HEMOSTATIC DRESSINGS
O-12	Rosana Nunes dos Santos	STUDIES FOR THE DEVELOPMENT OF POLYMERIC BIOABSORBABLE STENT FOR COARCTATION OF THE AORTA - COA
O-13	Daniel Canales Arevalo	BIOLOGICAL EVALUATION OF ELECTROSPUN FIBERS BASED ON POLYACRYLONITRILE/CALCIUM OXIDE NANOCOMPOSITES AS A BIOACTIVE SCAFFOLD FOR BONE TISSUE ENGINEERING
O-14	Milton Flávio de Macedo	USE OF BIOMATERIALS IN REGENERATIVE MEDICINE – OUTPATIENT CELLULAR THERAPY – NEW PROTOCOLS AND EQUIPMENT
O-15	Isadora Spadrezano	ANTITUMORAL EFFICACY OF GOLD NANOPARTICLES WITH POLYPHENOLS IN BREAST CANCER AND METASTATIC CELLS IN VITRO
O-16	Diego Eterno de Oliveira Mendonça	CHARACTERIZATION OF THE PLA-BIOAlSr GLASS COMPOSITE WITH POSSIBLE CANDIDATE FOR BONE AND TENDINOUS REGENERATION APPLICATIONS
O-17	Jean Mendes Nascimento	CHILD ROBOTIC REHABILITATION SYSTEM WITH ASSESSMENT OF MUSCLE STRENGTH PROGRESSION
O-18	Santiago Sarasola	STIFFNESS AND DIMENSIONAL ACCURACY EXPERIMENTAL VALIDATION OF METALLIC 3D PRINTED LATTICE STRUCTURES
O-19	Bruno Henrique Costa	PRODUCTION OF BIONANOCOMPOSITES OF CARBON AND DIETARY PROTEINS AS A PROMISING DRESSING MODULATOR OF INFLAMMATION IN BONE REPAIR
O-20	Maria A. Lopes	HYBRID STRUCTURES FOR ACHILLES' TENDON REPAIR
O-21	Sergio Martin Saldaña	NANOGEL-BASED ADVANCED THERAPEUTIC FOR NOSE-TO-BRAIN DELIVERY TO TACKLE OXIDATIVE STRESS
O-22	Murilo Álison Vigilato Rodrigues	ULTRATHIN COLLAGEN AND GELATIN FIBERS: BENIGN SOLVENTS TO PRODUCE POTENTIAL BIOMIMETIC BIOMATERIALS BY SOLUTION BLOW SPINNING

O-23	Antonio Arleques Gomes	MICROFLUIDIC CIRCUIT APPLIED TO THE CONCENTRATION OF 18F FOR THE PRODUCTION OF RADIOPHARMACEUTICALS
O-24	Erika Costa Alvarenga	BIOGEL WITH PIEZOELECTRIC NANOPARTICLES AS OSTEOINDUCTIVE BIOMATERIAL
O-25	William Henrique Oliveira de Souza	STUDY OF POWDER METALLURGY TECHNIQUE FOR OBTAINING MG-ZN SYSTEM ALLOYS FOR BIOMEDICAL APPLICATIONS
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O-27	Merari Tumin Chevalier	ELASTIN LIKE HYDROGELS FOR BRAIN REPAIR AFTER ICHEMIC STROKE
O-28	Isabela Louise Pereira Lopes	NEW BIOMATERIAL BASED ON DECELLULARIZED EXTRACELLULAR MATRIX FUNCTIONALIZED WITH NANOCHITOSAN TO ELABORATE BIODRESSING
O-29	Paulo Soares	SYNERGISTIC EFFECT OF PEO-POLYMER HYBRID COATINGS WITH NANOPARTICLES INCORPORATION FOR IMPROVED TRIBOLOGICAL, BIOACTIVITY, AND BACTERICIDAL PROPERTIES ON TITANIUM IMPLANTS
O-30	Lais Caroline Souza e Silva	USE OF ALGINATE HYDROGEL TO IMPROVE GAIT IN ANIMALS WITH OSTEOARTHRITIS
O-31	Daniel Prada	HOMOGENISATION OF TITANIUM SCAFFOLDS USING MICROCT AND FEM
O-32	Hernane Barud	BACTERIAL NANOCELLULOSE AS A NEW ALTERNATIVE FOR CELL CULTURE PLATFORM
O-33	Camila Lourdes Frosasco	BIOLOGICAL EVALUATION OF BIODEGRADABLE POLYMER (PLA/B-TCP) IMPLANTS FOR THEIR POTENTIAL APPLICATION IN BONE TISSUE ENGINEERING
O-34	Larissa Sabino dos Santos	PRODUCTION OF POLYCAPROLACTONE/CURCUMIN MEMBRANES AND ITS EFFECTIVENESS IN CELLS GROWTH, PH SENSITIVITY AND RELEASE OF CURCUMIN
O-35	Luís Alberto Loureiro dos Santos	DEVELOPMENT OF CALCIUM PHOSPHATE CEMENT COATINGS ON TI6AL4V
O-36	Octavio Santiago	IN VITRO AND IN VIVO COMPATIBILITY OF ELASTIN-LIKE RECOMBINAMER-BASED HYDROGELS
O-37	Viviane do Socorro da Costa	EFFECT OF ZINC IN THE PROCESS OF HIGH ENERGY MILLING OF POWDERS TO OBTAIN THE ALLOY OF THE MG-ZN SYSTEM AIMING FOR BIOMEDICAL APPLICATIONS
O-38	Alexis, Wolfel	A BIOINSPIRED STRATEGY FOR CHEMO-SELECTIVE BIO-FUNCTIONALIZATION OF POLYACRYLAMIDE HYDROGELS WITH CELL-ADHESIVE LIGANDS
O-39	Rafael Palma	CHARACTERIZATION OF PECVD THIN DLC FILMS ON ALUMINUM SUBSTRATE AND CELL ADHESION TEST
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O-42	Henrique Nunes da Silva	EVALUATION OF CHITOSAN/N-ACETYL-D-GLUCOSAMINE SUTURE THREADS IN WOUND HEALING IN RAT SKIN (RATTUS NORVEGICUS)
O-43	Clara Soubelet	RHEOLOGICAL AND CURING PROPERTIES OPTIMIZATION ZRO2/BIOACTIVE GLASS NANOCOMPOSITE PHOTOCURABLE SLURRIES
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O-45	Guilherme Barbosa Lopes Jr	COMPUTATIONAL HEMODYNAMICS ON AN AXIAL BLOOD PUMP FLOW
O-46	Conrado Ramos Moreira Afonso	IN-SITU ALLOYING THROUGH SELECTIVE LASER MELTING OF B TI-15NB ATOMIZED PURE POWDERS FOR IMPLANT APPLICATIONS
O-47	Rossana Thiré	POTENTIAL IODOPHOR-BASED WOUND DRESSINGS VIA SOLUTION BLOW SPINNING

O-48	Amato, Luis Eduardo Javier	ESTIMATION OF TRABECULAR BONE SOLID FRACTION THROUGH ULTRASONIC TRANSMISSION TESTS
O-49	Giovanna do Espiritu Santo	MANUFACTURE OF 3D BIOSILICA SCAFFOLDS FOR BONE TISSUE REPAIR: PHYSICAL-CHEMICAL EVALUATION
O-50	Cecília Zavaglia	ROTARY JET SPINNING – A VERSATILE TECHNIQUE: SCAFFOLDS OF PCL WITH BIOCERAMICS OR PHYTOTHERAPICS FOR TISSUE ENGINEERING
O-51	Patrícia Lima Falcão	ACTIVATION OF STAT-3 SIGNALLING BY UPREGULATION OF IL-6 AS POTENTIAL TARGET FOR DECREASING CLONOGENESIS OF RADIORESISTANT MDAMB-23
O-52	Ângela Maria Moraes	ASSESSMENT OF IN VITRO CYTOTOXICITY OF DIFFERENT HYDROGEL FORMULATIONS AND CROSSLINKING SOLUTIONS AIMING AT 3D BIOPRINTING APPLICATIONS
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O-54	Guilherme Barbosa Lopes Jr	LARGE EDDY SIMULATION APPLIED TO REACH CRITICAL ZONES IN A CENTRIFUGAL BLOOD PUMP SIMULATION
O-55	Alexis, Wolfel	HIGH RESOLUTION VOLUMETRIC BIOPRINTING OF LARGE ENGINEERED TISSUES
O-56	Wellington Luís	CHITOSAN FILMS RETICULATED WITH LACTIC ACID AND FUNCTIONALIZED WITH NANO CHITOSAN, AS A PROMISING BIOMATERIAL FOR BIODRESSINGS
O-57	Flavia Pedrini	UNLOCKING THE POTENTIAL OF POLY(L-CO-D,L-LACTIC ACID-CO-TRIMETHYLENE CARBONATE) FOR EXTRUSION-BASED 3D PRINTING
O-58	Marcelo Sá	ASSESSMENT OF PURE-PHASE B – TRICALCIUM PHOSPHATE (β – TCP) CYLINDRICAL CERAMIC IMPLANT OSSEOINTEGRATION USED IN CRITICAL SEGMENTAL BONE DEFECTS OF THE RADIUS OF RABBITS
O-59	Eduardo Bock	WIRE ARC ADDITIVE MANUFACTURING USING CONTROLLED SHORT CIRCUIT
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O-65	Marcelo Garrido dos Santos	PRODUCTION AND CHARACTERIZATION OF A BIOINK CONTAINING DECELLULARIZED SPINAL CORD TISSUE AND AN ELECTRICAL CONDUCTIVE POLYMER FOR 3D BIOPRINTING
O-66	Claudia Larissa Viana da Silva	INFLUENCE OF HUMAN TYPE I COLLAGEN ON THE PERFORMANCE OF ANIMAL-FREE BIOINKS FOR 3D BIOPRINTING: A COMPARATIVE STUDY
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O-68	Bibiana Madeira Melo	BIOMIMETIC STICKER GELATIN-BASED HYDROGEL FUNCTIONALIZED BY PHENOLIC COMPOUNDS
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Poster presentations

SESSION I – WEDNESDAY, DECEMBER 13

CODE	T	PRESENTING AUTHOR	ABSTRACT TITLE
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P-02	T1	Ailime Melo	DEVELOPMENT OF PELVIC FLOOR EDUCATORS THROUGH 3D PRINTING TECHNIQUE
P-03	T1	Carmen González Henríquez	3D PRINTING OF BIOCOMPATIBLE AND BIOACTIVE PHOTOCURABLE RESINS FOR APPLICATIONS IN BONE REGENERATION
P-04	T1	Mauricio Sarabia Vallejos	MECHANICAL IMPROVEMENT OF BIOCOMPATIBLE AND PHOTOCURABLE RESINS FOR 3D PRINTING FOR BONE REGENERATION USING ADVANCED MULTI-POROUS DESIGNS
P-05	T1	Rodrigo Cesar Gomes	STUDY OF IN VITRO DEGRADATION AND DRUG DELIVERY OF AN ENDONASAL MEDICAL DEVICE 3D PRINTED BASED IN PLDLA-TMC WITH MOMETASONE FUROATE
P-06	T1	Claudia Larissa Viana da Silva	3D BIOPRINTING OF FIBROBLASTS FOR THE PRODUCTION OF QUANTIS® BIOIDENTICAL HUMAN COLLAGEN: AN INNOVATIVE APPROACH IN ADDITIVE MANUFACTURING
P-07	T1	Marcelo B. Furtini	DEVELOPMENT OF 3D PRINTER FILAMENT INCORPORATED WITH OXIDE FOR ORTHOSES
P-08	T2	Tarciana Dieb Toscano	ASSESSMENT OF CORROSION RESISTANCE ON THE SURFACE OF NANOTEXTURED STAINLESS STEEL COATED WITH A THIN COPPER FILM FOR BIOMEDICAL APPLICATION.
P-09	T2	Viviane Costa	CHARACTERIZATION OF POWDER FROM THE MG-ZN SYSTEM OBTAINED BY POWDER METALLURGY FOR BIOMEDICAL APPLICATIONS
P-10	T2	Sinara Borborema	EFFECT OF THE MEDIUM ACIDITY ON THE CORROSION RESISTANCE OF TI-10MO-30NB ALLOY FOR BIOMEDICAL APPLICATIONS
P-11	T2	Tainara Lima	DEVELOPMENT OF MICROFIBRILLAR MEMBRANES BASED ON POLY(E-CAPROLACTONE) TO BE USED IN THE TREATMENT OF CUTANEOUS LESIONS
P-12	T2	Luiz Henrique de Almeida	STUDY OF THE CORROSION PROPERTIES IN FUNCTION NB ADDITION IN THE TI-10MO-XNB ALLOYS FOR BIOMEDICAL IMPLANTS
P-13	T2	Sergio A. Yoshioka	CHARACTERIZATION OF CRUDE CORN ZEIN MICROSPHERES OBTENINED BY ANTI-SOLVENT-DIALYSIS METHOD WITH/WITHOUT THE AID OF ULTRASOUND
P-14	T2	Karine Modolon Zepon	SANDWICH-LIKE FILM FOR ON-DEMAND TOPICAL RELEASE OF LIDOCAINE
P-15	T2	Karine Modolon Zepon	LIPASE-RESPONSIVE NANOPARTICLES FOR ANTIBIOTIC DRUG DELIVERY
P-16	T2	Mirian Bonifacio	CHARACTERIZATION OF CHITOSAN HYDROGEL FOR OSTEOARTHRITIS TREATMENT
P-17	T2	William Henrique Oliveira de Souza	STUDY OF HIGH-ENERGY MILLING AND SINTERING OF MG-ZN SYSTEM ALLOYS

P-18	T2	Thaíla Silva	PREFILLED SYRINGES WITH SILICONIZED SURFACES: AN OVERVIEW
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P-23	T2	Marcelo B. Furtini	OBTAINING ANTIBACTERIAL MATERIAL BASED ON CHLORHEXIDINE AND CASSAVA GUM
P-24	T2	Juliana de Moraes	STUDY OF THE DEGRADATION OF 3D PRINTED SCAFFOLDS BASED ON THE PLDLA-TMC TERPOLYMER IN DIFFERENT RATIOS FOR BIOMEDICAL APPLICATIONS.
P-25	T2	Luciana Malvestiti	MG ZX10 FOR PERIPHERAL NERVE REGENERATION: COMPARATIVE CORROSION STUDIES UNDER SIMULATED BIOLOGICAL CONDITIONS
P-26	T2	Marcelo Brito Madureira	DEVELOPING AND CHARACTERIZING A BIOABSORBABLE MEMBRANE TO COMBAT INFECTION IN ORTHOPEDIC IMPLANTS
P-27	T2	Cintia Cristina Santi Martignago	DEVELOPMENT AND CHARACTERIZATION OF HYDROGEL FOR USE IN KNEE JOINT DISORDERS
P-28	T2	Katherina Fernandez	MECHANICAL CHARACTERIZATION OF ALG/RGO/TA HYDROGELS
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P-30	T2	Luciara da Silva	MICROSTRUCTURAL ANALYSIS OF POLYCAPROLACTONE AND ATORVASTATIN NANOCOMPOSITES
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P-33	T2	Rodrigo Cesar Gomes	MECHANICAL CHARACTERIZATION, HYDROLYTIC DEGRADATION, AND CELL VIABILITY OF A NEW POLYURETHANE BASED ON PCL AND TRIMETHYLENE CARBONATE
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P-36	T2	Yuri Vieira Alves	ANALYSIS AND CHARACTERISTICS OF ADVANCED CERAMICS AS A BIOMATERIAL FOR ORAL AND MAXILLOFACIAL USE
P-37	T2	Diógenes Santos Dias	BIOPLASTIC BASED ON CUCUMBER MESOCARP PUREE (CUCUMIS SATIVUS)
P-38	T2	Eurico F. Pieretti	CORROSION ANALYSES OF ANODIZED ALUMINUM FOR BIOMEDICAL PURPOSES
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P-40	T2	Eurico Pieretti	MAGNETIC PROPERTIES EVALUATION OF 316L STAINLESS STEEL PRODUCED BY ADDITIVE MANUFACTURING FOR BIOMEDICAL USE
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P-42	T2	Jesualdo Luiz Rossi	PREPARATION AND CHARACTERIZATION OF TITANIUM-ZIRCONIUM ALLOYS
P-43	T2	Rita de Cássia Reis Rangel	INFLUENCE OF ANNEALING TEMPERATURES ON THE STRUCTURE AND WETTABILITY OF IRON OXIDE NANOTUBES
P-44	T2	Danielle Duque Domingues	HEMATITE SYNTHESIS BY POLYMERIC PRECURSORS METHOD
P-45	T2	Gabriel Clem Albuquerque Sasdelli	POLYVINIYL ALCOHOL MATS CROSSLINKED WITH CITRIC ACID FOR REGENERATIVE MEDICINE APPLICATIONS
P-46	T3	Rodrigo Silveira Vieira	NATIVE COLLAGEN EXTRACTION FROM TUNA SKIN BY ACID TREATMENT
P-47	T3	Jessica Asami	THE IMPACT OF NON-DEPROTEINIZATION ON PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF NATURAL RUBBER LATEX FOR BIOMEDICAL APPLICATIONS
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P-49	T3	Franco Leonardo Redondo	STUDY OF CELL VIABILITY OF COMPOSITE POLYMER COATINGS OBTAINED BY EPD METHOD AND POROUS SCAFFOLDS OF PLA MANUFACTURED BY 3D PRINTING
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P-52	T3	Ricardo Barbosa de Sousa	BACTERIAL CELLULOSE/CALCIUM PHOSPHATES COMPOSITE CONTAINING CERIUM FOR BONE TISSUE ENGINEERING APPLICATIONS
P-53	T3	Victor Marchiori de Souza	IMPACTS OF DIFFERENT STRATEGIES FOR THE DECELLULARIZATION OF PORCINE AURICULAR CARTILAGE AS MODEL SCAFFOLDS FOR TISSUE ENGINEERING
P-54	T3	Karolyne dos Santos Jorge Sousa	EVALUATION OF GENOTOXICITY AND CYTOTOXICITY OF BIOSILICA AND SPONGIN DERIVED FROM MARINE SPONGES FOR 3D PRINTED SCAFFOLD PRODUCTION
P-55	T3	Verónica Rodrigues Gonzaga	RECENT TECHNOLOGICAL ADVANCES IN THE PROCESSING OF CONSTRUCTS TO OBTAIN MEAT GROWN IN LABORATORIES: A TECHNICAL, ECONOMIC AND ENVIRONMENTAL FEASIBILITY STUDY (EVTEA)
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P-58	T3	Nadina Aimé Usseglio	WOUND TREATMENT: BIONANOCOMPOSITE FILMS TRANSFORMING FROM SOLID TO GEL STATE
P-59	T3	Rossemberg Cardoso Barbosa	BIODEGRADABLE SCAFFOLDS WITH JATROPHA MOLLISSIMA (POHL) BAILL EXTRACT. FOR USE AS DRESSINGS
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P-63	T3	Caio Canelas	DEVELOPMENT AND IN VITRO EVALUATION OF BIOMATERIALS PRODUCED BY ROTARY JET SPINNING BASED ON PCL AND PHB WITH ADDITION OF AMAZONIAN VEGETABLE OILS

P-64	T3	Francisco Eroni Paz dos Santos	SYNTHESIS OF A NEW ANTIBACTERIAL BIOMATERIAL CONTAINING HYDROXYAPATITE / CLORHEXIDINE FOR FUTURE APPLICATIONS IN BONE TISSUE ENGINEERING
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P-66	T3	Larissa Ribeiro Lourenço	CHITOSAN HYDROGEL CONTAINING BIOACTIVE GLASS AIMING AT 3D PRINTING FOR PERIPHERAL NERVE REGENERATION
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P-70	T3	Jaqueline Leite Vieira	INFLUENCE OF NHA PARTICLES SIZE ON THE PROPERTIES OF 3D PRINTED PLA-BASED SCAFFOLDS
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P-72	T3	Bruna Vanessa Quevedo	ELECTROSPUN MEMBRANE OF POLY(L-CO-D,L LACTIC ACID) AND NATURAL RUBBER CONTAINING COPAIBA OIL FOR ANTIMICROBIAL DRESSING DEVELOPMENT
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P-74	T3	Júlia Bünecker Cassel	HIGH STRENGHT BIOMIMETIC SILK FIBROIN/A-TRICALCIUM PHOSPHATE BONE CEMENT WITH CHLORIDE-SUBSTITUTED HYDROXYAPATITE
P-75	T3	Marcelo Garrido dos Santos	PRODUCTION AND CHARACTERIZATION OF A BIOINK FOR 3D BIOPRINTING CONTAINING DECELLULARIZED SKIN AS A POTENTIAL DERMAL SUBSTITUTE
P-76	T3	Oriana Perna	PARTIALLY AND FULLY HYDROLYSED POLYVINYL ALCOHOL CROSSLINKED WITH HEXAMETHYLENE DISSOCIATE AS A BONE SCAFFOLD.
P-77	T3	Bruna Nunes Teixeira	SPINNABILITY OF POLYCAPROLACTONE FIBERS LOADED WITH STRONTIUM-MODIFIED HYDROXYAPATITE
P-78	T3	Diego Eterno de Oliveira Mendonça	PLA-BIOAlSr GLASS COMPOSITE AS A CANDIDATE FOR BONE AND TENDINOUS REGENERATION APPLICATIONS

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P-80	T3	Michelle Oliveira	DEVELOPMENT AND CHARACTERIZATION OF COLLAGEN TYPE I BIOCOMPOSITES REINFORCED WITH 2D NANO MINERALS FOR BIOMEDICAL APPLICATIONS
P-81	T3	Priscila Randazzo-Moura	THE PHARMACOLOGICAL EFFECTS OF ARNICA EXTRACT INCORPORATED INTO PVA [POLYVINYL ALCOHOL)] IN A DELIVERY SYSTEM ASSOCIATED WITH LOW-INTENSITY LASER
P-82	T3	Priscila Randazzo-Moura	IN VITRO STUDY OF MANGANESE (MN2+) ASSOCIATED WITH HYALURONIC ACID ON THE CYTOTOXICITY OF BOTHROPS JARARACUSSU VENOM
P-83	T3	Eliana Aparecida de Rezende Duek	PHARMACOLOGICAL EFFECTS OF TRIAMCINOLONE ASSOCIATED WITH SURGICAL ADHESIVE ON CUTANEOUS WOUND HEALING IN WISTAR RATS

P-84	T3	Melanie Ferrace	STUDY OF BIORESORBABLE POLYMERS THERMAL STABILITY FOR FIBER PRODUCTION
P-85	T3	Bianca Sabino Leocádio Antunes	RHEOLOGICAL CHARACTERIZATION OF SILK FIBROIN/PLDLA/SIMVASTATIN-BASED GEL FOR 3D PRINTING.
P-86	T3	Mercedes Pérez-Recalde	DEVELOPMENT OF A COLLAGEN AND CHITOSAN BIOINK
P-87	T3	Willams Teles Barbosa	DEVELOPMENT OF BIOCOMPOSITE MEMBRANES OF BACTERIAL CELLULOSE AND HYDROXYAPATITE FOR APPLICATION IN BONE TISSUE
P-88	T3	Willams Teles Barbosa	3D BIOPRINTING AND CHARACTERIZATION OF SCAFFOLDS WITH DIFFERENT CONCENTRATIONS OF GELATIN METHACRYLATE (GELMA) FOR SKIN WOUND REPAIR
P-89	T3	Alejandra Fanovich	POROUS TITANIUM DIOXIDE-HYDROXYAPATITE COMPOSITES PREPARED BY A SOL-GEL/SUPERCRITICAL CO2-DRYING COMBINED PROCESS.
P-90	T3	Matthäus Davi Popov Pereira da Cunha	CORE-SHELL ELECTROSPUN NANOFIBERS BASED ON SOY PROTEIN ISOLATE FOR TISSUE ENGINEERING APPLICATIONS
P-91	T3	Pablo Botta	DEVELOPMENT OF MAGNETIC BONE CEMENT BASED ON CALCIUM PHOSPHATE AND IRON OXIDE NANOPARTICLES
P-92	T3	Edson Cavalcanti Silva-Filho	DEVELOPMENT OF A NOVEL CASHEW TREE GUM CONTAINING HYDROXYAPATITE SCAFFOLD FOR BONE REPAIR PURPOSES
P-93	T4	Eduarda Baggio Paglia	SELECTIVE CAPTURE OF PROSTATE TUMOR CELL LINES ON MULTILAYERED FILMS OF CHITOSAN AND HYALURONIC ACID
P-94	T4	Gabriela Pereira de Freitas	EFFECTS OF PH AND SALT CONCENTRATION ON THE SURFACE PROPERTIES OF MULTI-LAYER FILMS BASED ON BIOPOLYMERS FOR MEDICAL TUMOR CELL CAPTURE DEVICES
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P-96	T4	Karolyne dos Santos Jorge Sousa	IN VITRO STUDY OF 3D PRINTED SKIN DRESSING FROM MARINE COLLAGEN
P-97	T4	Daniel Ribeiro	IN VITRO GENOTOXICITY AND CYTOTOXICITY ANALYSIS OF BIOGLASS/SPONGIN SCAFFOLDS FROM MARINE SPONGES FOR BONE TISSUE ENGINEERING APPLICATIONS
P-98	T4	Ariadna Birocco	DERMATAN SULFATE/CHITOSAN POLYELECTROLYTE COMPLEXES FOR THE DELIVERY OF FLUBENDAZOLE TOWARDS TRIPLE NEGATIVE BREAST CANCER CELLS
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P-149	T11	Adriana de Souza Rodrigues	SILVER NANOPARTICLES REDUCED BY TANNIC ACID AND SODIUM CITRATE: A SYNERGIC APPROACH WITH ANTIMICROBIAL PROPERTIES
P-150	T11	Sofía Genovés	DEVELOPMENT OF IMMUNOMODULATORY NANOCOMPLEXES (NANOIMMUNOMODULATORS-NIMS) AGAINST PATHOGENIC BACTERIA
P-151	T11	Taynah Pereira Galdino	CHITOSAN NANOPARTICLES WITH RUTIN AS A POTENTIAL THERAPEUTIC SYSTEM IN THE TREATMENT OF COVID-19.
P-152	T11	Rosane Soares	GALACTOMANNAN-BASED HYDROGEL AS NANOCARRIER OF α-BISABOLOL FOR WOUND HEALING APPLICATION
P-153	T12	Arielly da Hora	DEVELOPMENT OF MICROFLUIDIC DEVICE FOR ESTABLISHMENT OF THE GLIOBLASTOMA-ON-A-CHIP

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TOPIC 1

Additive manufacturing (including Biofabrication)

RHEOLOGICAL AND CURING PROPERTIES OPTIMIZATION OF ZrO_2/BIOACTIVE GLASS NANOCOMPOSITE PHOTOCURABLE SLURRIES

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Keywords: Masked stereolithography, zirconia, bioactive glass, scaffolds

Introduction and objective: Materials demand for biomedical applications has had a sustained growth worldwide. Stereolithography is a manufacturing technology that can replace other high costs shaping methods such as CAD/CAM or injection molding. Using common commercially available printers is challenging for manufacturing ceramics. Nevertheless, they could be used if an adequate rheological behavior of photocurable ceramics slurries is achieved using the right type of dispersant [1]. The main objective of this investigation is to optimize the rheology and the curing properties to develop high solid loaded resins of 3 mol% Y_2O_3 stabilized tetragonal zirconia (3Y-TZP) particles coated with a sol-gel bioactive glass.
Methodology: Ceramic suspensions were prepared using a resin composed by Acryloymorpholine (ACMO), 2-Phenoxyethyl acrylate (2-PEA) and Pentaerythritol [5 EO] tetraacrylate (PPTTA) monomers with low initial viscosity. Optimum dispersant concentration was determined by measuring viscosity of 40 vol% sol-gel bioactive glass (64% SiO_2, 26% CaO, 10% P_2O_5, mol%) coated ZrO_2 particles suspension. Different photoinitiator (diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide - TPO) concentrations were studied to obtain the highest achievable curing depth. Curing experiments were carried on at different exposure times to determine penetration depth and critical exposure energy. Finally, curing parameters were used to define the appropriate exposure time to print 10 µm thickness layers.
Results and discussion: A decreasing trend behaviour was found with the increase in the dispersant concentration up to 5 wt% (900 mPa.s at ~1 s^{-1} shear rate), followed by an increase with further dispersant additions. The highest curing depth was found with the addition of 0.5 wt% of TPO, at the whole range of exposure times studied. Curing parameters were fitted according to the modified Beer-Lambert law, founding D_p= 15.3 µm and E_c = 2.09 mJ/cm^2. Taking into account these parameters, it was possible to calculate the exposure time needed to print 10 µm layers, considering an excess curing factor of 3 to ensure a good adherence between layers. The exposure time needed was 4 seconds.
Conclusions: Viscosity properties and curing parameters were studied. Optimum dispersant concentration allowed obtaining a low viscosity suspension suitable for a homogeneous and defect-free printing. With the curing parameters found, it is possible to use common commercial 3D printers to create custom shaped ceramics.

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IN-SITU ALLOYING THROUGH SELECTIVE LASER MELTING OF β Ti-15Nb ATOMIZED PURE POWDERS FOR IMPLANT APPLICATIONS

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Keywords: β-Ti alloys, In-Situ Alloying; Biomaterials; Additive Manufacturing

Introduction and objective: β Ti alloys are acclaimed for biomedical applications due to their mechanical properties, good biocompatibility and corrosion resistance. Based on these characteristics, pure Ti and different Ti alloys have been applied in the biomedical industry, especially the Ti-6Al-4V alloy. However, although it presents good mechanical properties, this alloy presents problems regarding cytotoxicity of Al and V elements related to neural disorder and Alzheimer's disease. Furthermore, for orthopedic applications, there is a considerable difference between elastic modulus of Ti and α+β alloys, from E = 100 to 110 GPa, and that of human bone, around E = 30 GPa. The objective of this work is study and characterize metastable β Ti-15Nb alloy (wt.%) processed through laser powder bed fusion (LPBF) and arc melting, since this is free of cytotoxic elements and shows lower Young modulus.
Methodology: Mechanical mixture of pure powder of Ti and Nb was done in the proportion Ti-15Nb (wt.%), using a LPBF equipment OmniTek, model OMNISINT-160 with fiber laser Yb:YAG, Raycus RFL-C500, and wavelenght λ=1070 nm, spot size of Ø = 80 µm, a layer thickness of t = 30 µm and a hatch space of 80 µm. Characterization of LPBF β Ti-15Nb alloy and copper mold cast alloys were done by optical microscopy (OM), X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission (TEM) coupled to ASTAR - automatic crystal orientation mapping (ACOM). Vickers microhardness, and elastic modulus via nanohardness and impulse excitation were evaluated.
Results and discussion: SLM of β Ti-15Nb alloy showed a α´+ β microstructure, the Vickers microhardness and elastic modulus measured by nanohardness and impulse excitation technique were evaluated, showing suitable modulus of around 60 GPa for biomedical applications. Besides that, the microhardness was found around 300 HV and density, which plays an important role in mechanical properties, was found above 99%. Therefore, SLM of β Ti- 15Nb has been showing a promising material for orthopaedic applications. The phases found in Ti-15Nb samples manufactured by SLM using a mixture of elemental metallic powders were α' and β and X-ray diffraction analysis have not detected precipitation of ω phase. Furthermore, it was not possible to found a clear relationship between the energy density and mechanical properties, which points that this parameter is not enough to describe and understand the SLM in-situ process. Regarding the Vickers hardness, the results are controlled by the selected processing conditions, the lowest hardness found was 298 HV while the lowest elastic modulus found was 59 GPa.

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MANUFACTURE OF 3D BIOSILICA SCAFFOLDS FOR BONE TISSUE REPAIR: PHYSICAL-CHEMICAL EVALUATION

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Keywords: 3D printing, biosilica, bone repair, marine sponges.

Introduction and objective: The increasing occurrence of critical bone fractures affects approximately 200 million people worldwide, resulting in high costs for proper treatment. Therefore, the use of bioceramics is an accessible and viable alternative, among which the use of biosilica extracted from marine sponges can be mentioned, given its proven osteogenic effects in *in vitro* and *in vivo* studies [1]. To optimize scaffold manufacturing, 3D printing techniques have enabled the customization of their three-dimensional structures, promoting cell adhesion and proliferation. Thus, the objective is to manufacture 3D scaffolds using a grid model of marine biosilica and evaluate their physicochemical characteristics and biological efficacy in *in vitro* assays.
Methodology: For the physicochemical analyses, pH tests, mass loss, and calcium assay in Simulated Body Fluid (SBF) were performed. Subsequently, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-ray Spectroscopy (EDS), and apparent porosity assay were conducted to analyze the composition, mechanical properties, and surface of the scaffolds. Statistical analysis of the results was performed using Two-Way ANOVA and POST-HOC Bonferroni test, with a significant difference set at $p < 0.05$.
Results and discussion: As a result, the model showed acidification in the first 14 days, and on day 21, there was an increase to an average pH of 5.046 ± 0.030. In mass loss, controlled degradation was observed, reaching 84% ± 2.42. In the calcium assay, the model exhibited better control of this behavior over the experimental time [2]. The final average percentage of porosity reached 85.89% ± 0.9256. FTIR analysis revealed characteristic peaks of biosilica and alginate. SEM showed more evident pores in the grid model, as well as the filament. EDS results indicated the presence of Si, Ca, and Cl elements over the experimental time, derived from the interaction with SBF fluid and the printing ink production process.
Conclusions: Based on the results, there is potential for conducting *in vitro* assays with MC3T3-E1 (osteoblasts) and L929 (murine fibroblasts) cell lines to evaluate cell adhesion, proliferation, and mineralization. Additionally, compression testing can be performed to understand the mechanical behavior and the interaction between biosilica from marine sponges and sodium alginate polymer.

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ASSESSMENT OF *IN VITRO* CYTOTOXICITY OF DIFFERENT HYDROGEL FORMULATIONS AND CROSSLINKING SOLUTIONS AIMING AT 3D BIOPRINTING APPLICATIONS

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Keywords: biopolymers, crosslinking solutions, cytotoxicity, 3D bioprinting.

Introduction and objective: Hydrogels of biopolymers have been employed as matrices for 3D bioprinting, since they may enable cell proliferation, adhesion, and migration. To enhance their mechanical strength, cellulose particles and polysaccharide crosslinking agents are commonly added. However, it is crucial to ensure these reinforcing methods are not harmful to the cells. This study aimed to identify noncytotoxic concentrations of cellulose nanocrystals (CNCs) and $CaCl_2$ or $SrCl_2$ crosslinking solutions suitable for bioprinting using polysaccharide-based hydrogels and dental pulp stem cells (DPSCs) as bioink components, and to analyze the properties of resulting constructs.
Methodology: Either xanthan gum or hyaluronic acid were added to an alginate/HEPES buffer solution at a 3:1 mass ratio. The hydrogels were reinforced with CNCs at a 12:1 ratio to the alginate concentration, and their rheological behaviour was evaluated. The most adequate formulations and the crosslinking solutions were tested regarding *in vitro* cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The resulting bioprinted constructs were then characterized regarding cellular metabolic activity, filament width and height, pore size, fidelity of the final geometry to the designed structure, mechanical properties and stability.
Results and discussion: Hydrogels composed of alginate, xanthan gum (or hyaluronic acid), and CNCs at 1:3:12 mass ratio fulfilled the printability criteria. Rheological analysis demonstrated gel-like properties ($\tan \delta > 1$, ω = 10 to 100 rad.s^{-1}) and shear-thinning behaviour for these formulations (Ostwald-de-Waele model, $n < 1$). DPSCs cell proliferation was not significantly affected by the hydrogels' extracts (metabolic activity remained above 80%). The effects of prolonged contact (up to 24 h) between the biomaterials and solutions of the crosslinking agents was investigated aiming at maintaining bioprinted construct stability during tissue maturation periods, showing that $CaCl_2$ led to a metabolic activity decrease of over 49% at the lowest concentration (0.1M) and up to 70% at the highest (0.2M). Conversely, $SrCl_2$ at 0.1M maintained metabolic activity above 80%, ensuring post- printing stability and shape fidelity of the constructs.
Conclusions: In conclusion, either xanthan gum-or hyaluronic acid-alginate hydrogels, mechanically reinforced with cellulose nanocrystals, can be successfully associated with DPSCs for 3D bioprinting applications. Moreover, the crosslinking of these hydrogels using 0.1M $SrCl_2$ solution does not compromise cell viability, making it suitable for maintaining construct mechanical stability.

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HIGH RESOLUTION VOLUMETRIC BIOPRINTING OF LARGE ENGINEERED TISSUES

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Keywords: volumetric bioprinting; xolography; dual-color photoinitiators; multiscale structures

Introduction and objective:
Large tissues demand intricate and multiscale (hierarchical) architectures to be functional. For example, vascular networks, having channels with diameters that span from the centimetre (e.g., veins) to the micrometre scale (e.g, capillaries), are fundamental for the survival and function of virtually all tissues and organs. However, current biofabrication tools are not able to reproduce such multiscale architectures in a reliable and scalable manner and within a practical time window [1]. Very recently, a revolutionary volumetric printing technique was invented. Xolography can print plastics with unprecedented speed and resolution [2]. In this work, we optimized xolography to enable rapid and high-resolution bioprinting of cell-laden hydrogels.

Methodology:
Xolography was originally designed for the printing of hydrophobic plastic resins and requires using novel dual color photoinitiators (DCPIs), and co-initiators such as triethanolamine (TEA). To optimize xolography for bioprinting, we first characterized the cytotoxicity of DCPIs and TEA, and their photophysical properties in aqueous solutions. Following, we screened different photopolymerizable materials (e.g. GelMA and vinylic monomers) to define optimal formulations for 3D-printing of hydrogels while optimized parameters such as components concentration; printing speed; and energy of the light sources. We compared the printing of cell- free and cell-laden hydrogels and evaluated cell viability after printing.

Results and discussion:
DCPIs showed high cell viability (>98%) at the concentrations required for 3D-printing. Since DCPIs are a fundamental part of xolography, these results are a key aspect to confirm the potential of xolography as a tool for biofabrication. However, TEA displayed concentration-dependent cytotoxicity, prompting us to define upper bound concentrations for its use (<5 wt%). Within these boundaries, we developed different formulations for 3D-printing by combining GelMA and vinylic comonomers. The incorporation of comonomers into pure GelMA formulations played a crucial role by increasing the crosslinking speed, thus enabling the first reported xolographic printing of hydrogels. In less than 3 minutes, we printed cm-scale structures with features below 100 µm resolution, along with architectures presenting perfusable interconnected channels, thus demonstrating the high speed, resolution, and versatility of this new technology. In addition, 3D-bioprinted cell-laden hydrogels showed adequate cell viability, emphasizing the potential of bioxolography for the rapid biofabrication of large- scale tissue models.

Conclusions:
Bioxolography is presented as a next generation technology for volumetric bioprinting of tissue engineered constructs. This seminal work demonstrates that this technology is suitable for the 3D-bioprinting of cm-scale hydrogels at high speeds and resolutions with high cell viability. Bioxolography holds the potential to bioprint multi-scale architectures within practical time windows, thus offering a solution to an important and long- standing challenge in large-scale biofabrication of living matter.

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UNLOCKING THE POTENTIAL OF POLY(L-co-D,L-LACTIC ACID-CO-TRIMETHYLENE CARBONATE) FOR EXTRUSION-BASED 3D PRINTING

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Keywords: 3D printing; rheology; viscoelasticity; tissue engineering

Introduction and objective: Extrusion-based 3D printing is a well-established material processing technique that has shown promise in employing a variety of polymers. Poly(lactic acid) is the most studied and widely used raw material in 3D printing, which can be modified by copolymerization with trimethylene carbonate (TMC). However, the knowledge about the rheological properties of the Poly(L-co-D,L-lactic acid-co-trimethylene carbonate) (PLDLA-TMC) terpolymer remains limited. In light of this, this work aims to explore the characterization of PLDLA-TMC at different ratios (60/40, 70/30, 80/20, and 90/10), with a primary focus on rheological properties, printability, and cytocompatibility.
Methodology: The steady-state viscosity (η) as a function of shear rate ($\dot{\gamma}$) and the oscillatory rheological measurements of PLDLA-TMC 60/40, 70/30, 80/20, and 90/10 were performed in a DHR-2 rheometer (TA instruments) with a parallel plate geometry (25 mm of diameter). The scaffolds of these materials were printed using a 3D bioprinter (Octopus™) and for each PLDLA-TMC ratio, printing parameters were determined by conducting printability tests at different temperatures. The morphological analysis of the scaffolds was performed by scanning electron microscopy. Finally, mesenchymal stem cells were used to study the cytocompatibility of the printed PLDLA-TMC scaffolds using laser scanning confocal microscopy.
Results and discussion: The results demonstrated the shear-thinning behavior of PLDLA-TMC terpolymers, which meets the requirements for 3D extrusion-based printing [1]. The oscillatory measurements clearly show the influence of TMC content on the viscoelastic properties, with a predominantly liquid-like behavior being observed with increasing temperature. Additionally, the multi-mode Maxwell model provided a good fit to the viscoelasticity experimental data. [2]. The printing speed of 4 mm s^{-1} allowed the obtaining of scaffolds with rectangular pores, while controlling the extrusion temperature promoted the formation of uniform filaments with high printing quality, as observed by scanning electron microscopy. Confocal micrographs demonstrated that all PLDLA-TMC terpolymer ratios were able to promote MSC adhesion and proliferation after 7 and 21 days. Cells exhibited a spreading morphology characterized by clearly defined actin stress fibers throughout the scaffolds, with cell nuclei found up to 300 µm.
Conclusions: The outcomes obtained here reveal that the PLDLA-TMC terpolymer emerges as a promising candidate material for tissue engineering approaches based on 3D extrusion printing. The possibility of optimizing synthesis and printing parameters to enhance the rheological performance and printability of PLDLA- TMC allows for its application in a wide range of purposes.

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ADDITIVE MANUFACTURING PROCESS UTILIZING CONTROLLED SHORT-CIRCUITING

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Keywords: WAAM; CMT, additive manufacturing, controlled short circuit.

Introduction and objective : "WAAM (Wire Arc Additive Manufacturing) is the manufacturing process that refers to additive manufacturing using welding technologies, and operates on the principle of creating parts through layer deposition, using one of the technologies derived from the principle known as "Directed Energy Deposition" (DED) [1]. Technological advancements in energy sources, manipulation systems, and automation have significantly progressed WAAM, resulting in cost reduction and increased deposition rates of additive materials, enabling the production of components with varied dimensions for diverse technological sectors. The objective of this article is to demonstrate the evolution of this technology in the current industry and the quality of the deposits.
Methodology : The analysis focuses on the quality of additive manufacturing when performed on steel using a metal deposition process known as "DED" (Directed Energy Deposition). The technology employed was arc welding with the variant process of metal transfer known as "Cold Metal Transfer" (CMT) [2]. Two test specimens were fabricated: in the first, welding was conducted continuously without interruption between the deposited layers, while in the second, the deposition was paused for a duration of 20 seconds. This change in the process resulted in a significant variation in the wall of the manufactured substrates.
Results and discussion : The results achieved in the fabrication of the samples emphasize the importance of thermal control in the process of depositing multiple layers, utilizing WAAM technology. The produced geometries exhibit dimensional variations between the layers. Moreover, it is essential to thoroughly assess the visual quality of these layers in order to comprehend the heat transfer behavior to the substrate. Discrepancies in the final appearance of the manufactured substrate are also observed based on the pause time of each sample, even when the same welding parameters and robot displacement speed are applied.
Conclusions : WAAM, in the CMT variant, emerges as a viable technology for the production of components that require unconventional manufacturing methods. This study emphasizes that, even with the use of advanced DED technologies and stringent process control, it is crucial to manage heat transfer adequately during fabrication. This control can be achieved by interrupting the deposition process, as evidenced in this research, ensuring the homogeneity of the deposited layers using this technique.

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CAPES.

CELL INTERACTIONS WITH 3D PRINTING PLA/STRONTIUM-SUBSTITUTED HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS

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Keywords: PLA; hydroxyapatite; cytotoxicity; 3D printing

Introduction and objective: Bone fractures can be treated using the approach of tissue engineering, which proposes the use of scaffolds (structures to support new tissue), signalling molecules, and cells [1]. The strontium-substituted hydroxyapatite (HASr) is a promising ceramic material used to improve the biological properties of poly(lactic acid) (PLA) scaffolds [2]. The goal of this work was to evaluate the biological response to PLA/HASr scaffolds, produced by 3D printing, for Bone Tissue Engineering applications.

Methodology: PLA/HASr filaments were obtained using a single-screw extruder (0, 5, and 10% m/m). Scaffolds were manufactured by Fused Filament Fabrication (FFF) with the produced filaments and using digital files from previous authors' work [1]. Scaffolds were treated with NaOH solution (0.01 M) at 50°C for 15 min and sterilized with ethylene oxide (EtO). The biological response to the material was conducted with MC3T3 cells. The cell viability was assessed by LIVE/DEAD® (Viability/Cytotoxicity Kit - Invitrogen assay) after 24h in culture. The cell adhesion was analysed with Scanning Electron Microscopy (Tescan – Vega3) which allows seeing the cells' morphology.

Results and discussion: The scaffolds with irregular geometry were successfully produced by 3D printing. LIVE/DEAD images indicate that the material was not toxic for M3CT3 cells and that they were adhered to the surface of them. After 24 hours of incubation, cells were more spread onto the surface of PLA/HASr5 and PLA/HASr10 groups, in comparison with PLA/HASr0. The highest amount of HASr seems to improve the morphology of cells deposited onto the surface of PLA scaffolds and this result is in agreement with the literature [2]. The SEM micrographs corroborate the LIVE/DEAD results and indicate that cells are completely adhered and spread onto the surface of PLA/HASr scaffolds

Conclusions: The PLA/HASr scaffolds were successfully produced using the 3D Printing FFF technique. The cytotoxicity essay indicates no toxicity in the material. The cells show a good interaction with the biomaterial surface.

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PRODUCTION AND CHARACTERIZATION OF A BIOINK CONTAINING DECELLULARIZED SPINAL CORD TISSUE AND AN ELECTRICAL CONDUCTIVE POLYMER FOR 3D BIOPRINTING

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Keywords: Decellularization; 3D boprinting; Bioink; Neural tissue.

Introduction and objective: Over the last years, 3D bioprinting has emerged as a promising approach in the field of regenerative medicine. This technique allows for the production of three-dimensional scaffolds that support cell transplantation due to their ability to mimic the extracellular environment [1]. This feature is fundamental to support functional tissue regeneration [2]. To enhance cell adhesion, survival and proliferation, decellularized extracellular matrix can be used as a bioink component [3]. The aim of this study was to produce a bioink using lyophilized rat Decellularized Spinal Cord Tissue (DSCT) and PEDOT:PSS, a conductive polymer, for nervous tissue 3D bioprinting.

Methodology: Rat spinal cord tissue was decellularized using ionic detergents and freeze-dried. The bioink was produced with 1.5% DSCT, 3% gelatin, 4% alginate, 0.1 mg/mL PEDOT:PSS and $1X10^6$ mesenchymal cells/mL. Electrical conductivity was evaluated using a conductivity meter. Rheological characterization was performed using a rheometer with the Peltier equipment. Hydrogel SEM images were acquired using an electronic microscope. Cell viability was analysed using MTT and live/dead assays. The bioprinted cells were submitted to a neural differentiation protocol and stem cell and neural markers were analysed by flow cytometry.

Results and discussion: PEDOT:PSS addition to the hydrogel increased its electrical conductivity. The hydrogel presented shear thinning behaviour and low G''/G' ratio, allowing good printability without significantly compromising cell viability. DSCT presence in the hydrogel decreased gelatin thermal instability. SEM images showed that the biomaterial presented a highly porous tri-dimensional structure. MTT assay at day 1 after bioprinting indicated no viability reduction compared to the control. Live/Dead assay showed more than 75% cell viability 1 week after bioprinting. Flow cytometry indicated a decrease in the stem cell markers CD11b, CD44 and CD31 in the cells submitted to the differentiation protocol when compared to non-differentiated stem cells. Differentiation also induced an increased βIII-tubulin and glial fibrillary acidic protein (GFAP) expression.

Conclusions: According to the data mentioned above, the bioink presented optimal viscoelastic behaviour for 3D bioprinting. The material presented a porous internal structure that is fundamental to maintain cell viability, as indicated by MTT and live/dead assays. Furthermore, the bioprinted material supported neural differentiation. Therefore, DSCT and PEDOT:PSS containing bioink may be an easily-available biomaterial for neural tissue engineering via 3D bioprinting.

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PROMISING PULLULAN-REINFORCED POLYLACTIC ACID BIOCOMPOSITES FOR 3D PRINTING APPLICATIONS

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Keywords: Poly(lactic) acid; Pullulan; Biocomposites; 3d printing

Introduction and objective: Continuous advances in 3D printing technology have driven the manufacture of new biofilaments for biomedical applications [1]. This technology compared with conventional technologies presents low-cost, simple technology, and high efficiency rapid prototyping [2]. This paper aims to study the effect of using different pullulan volume ratios on the chemical, thermal, mechanical, and morphological properties of PLA filament for 3D printing.
Methodology: Filaments of poly(lactic) acid (PLA) filled with 10 and 5 wt% of pullulan were prepared in a thermokinetic mixer and extruded in a machine with to 180 °C and a screw rate of 7 rpm. The different temperatures for each composition were employed due to the dispersed pullulan loading amidst the polymer matrix, which interfered with the fluidity of the material as it passed through the heated extrusion chamber. The addition of pullulan significantly influenced the filament's diameter and density compared to pure PLA. Also, the thermal stability and morphological analysis were influenced by the addition of pullulan in the matrix.
Results and discussion: FTIR results indicated that pullulan altered the polymeric chain organization, impacting the polymer crystallinity as corroborated by DSC analysis. The biocomposites showed higher heat resistance in the range of 5–30% of mass loss, and all samples showed adequate thermal stability for fused deposition modeling (FDM) processing. The filaments developed have shown enhancement in their mechanical properties (shore hardness). The filament density showed no considerable variation within formulations. On the other hand, the diameter presented a considerable change based on the matrix used. The printed scaffolds represent novel biocomposites to the tissue regeneration field, being appropriate for biomedical and other 3D printing applications.
Conclusions: Despite the low concentration of pullulan on the composites investigated, the successful obtainment of PLA reinforced with pullulan, compromising neither its thermal properties nor its processability and printability, opens the possibility for future work investigation into a composite with larger pullulan content.

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DEVELOPMENT OF PELVIC FLOOR EDUCATORS THROUGH 3D PRINTING TECHNIQUE

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Keywords: polymers; biomaterials; physical therapy; pelvic floor educator.

Introduction and objective: Training on the female pelvic floor is very important for preventing pelvic muscle problems, such as urinary incontinence, organ prolapse, and pain during sexual intercourse. Some therapeutic devices, such as pelvic floor educators, are used to strengthen and stretch the muscles and tissues in this region. Thus, they assist in the therapy of pelvic dysfunctions through movement. However, in Brazil, the accessibility to these resources is limited due to high costs. This work aimed to develop a low-cost pelvic floor educator through a 3D printing technique. Two types of filaments were evaluated: polylactic-acid and flexible polyurethane.
Methodology: Prototypes were developed by defining an adequate anatomic model for this type of application, and characteristics such as comfort, size, and motility were considered. The prototype modeling was made via adaptations of pre-existing devices on the market. They were developed in Fusion360 software, from Autodesk. The pelvic floor educator was printed with Ender 3D printer from Creality using the fused filament fabrication technique.
Results and discussion: Three models were printed in polylactic acid (PLA) and flexible polyurethane (TPU). PLA models demonstrated high hardness and texture. Hardness is ideal for this type of application only if the model is anatomically correct. And the texture can lead to discomfort in the vaginal canal. TPU models showed greater motility than PLA due to their flexibility. This behaviour associated with the anatomic design of TPU models will allow, therefore, proprioception [1] due to the similarity of vibrators. Moreover, with this characteristic, the woman will easily assimilate and recognize the specific muscle.
Conclusions: To summarize, TPU educators showed greater motility than the printed PLA models, which are a thinner and more flexible intravaginal device. However, new adjustments to the design and an evaluation of pelvic physiotherapy are important in order to avoid device discomfort and better vaginal coupling.

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3D PRINTING OF BIOCOMPATIBLE AND BIOACTIVE PHOTOCURABLE RESINS FOR APPLICATIONS IN BONE REGENERATION

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Keywords: Bone scaffolds; Additive manufacturing; Bioactive fillers; PET-RAFT synthesis.

Introduction and objective: Tissue engineering (TE) emerges as a promising field aiming to integrate the design and development of cellular scaffolds, fostering bone regeneration and complete functionality restoration. Notably, the increasing adoption of additive manufacturing (AM) has revolutionized medical device production, facilitating the creation of adaptable techniques and strategies tailored to meet patients' unique needs, presenting a promising future for personalized medical interventions [1]. In this study, we aim to develop a new resin for 3D printing focused on bone regeneration applications. The improved performance of the resin is related to the interactions produced between the osteochondral cells and the material due to chemical modifications and the addition of bioactive ceramics in the resin.

Methodology: Three-dimensional scaffolds with internal microchannels were manufactured through PET-RAFT and DLP technology [2]. The biocompatible resins were manufactured using $PEGDA_{250}$ as a crosslinking agent, AAm, DMAEMA, HEMA, and HPMA as monomers, DBTTC and CDTPA as RAFT agents, in addition to Eosin Y (EY) and Rose Bengal (RB) as photoinitiator and photoabsorber, respectively. To increase the biocompatibility and mechanical strength of the bone scaffolds, nanohydroxyapatite (nHA) and β-tricalcium phosphate (β-TCP) were produced in the lab and introduced to the monomeric mixture as bioactive agents.

Results and discussion: Once the resin was synthesized and the samples were printed, biological, morphological, chemical, rheological, and mechanical approaches were used to assess and characterize the printability and properties of the micro-channeled scaffolds. FT-IR and Raman spectroscopies were used to determine the chemical structure of the printed material. Viscosities and surface tension of the resins were also measured, together with their contact angle with the printing plate. FE-SEM and EDS images were also obtained. Finally, cell culture tests were performed for the samples using osteochondral lines to ensure material biocompatibility. The findings show that adding bioactive agents improves the material's mechanical and bioactive capacities while maintaining the biocompatibility of the PET-RAFT resin.

Conclusions: Finally, due to its biocompatibility, mechanical resilience, and bioactive capability, the resin produced by our research group has the potential to be employed as a basis material for bone scaffolds. The PET-RAFT resin was improved to increase biocompatibility levels similar to commercial resins on the market.

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MECHANICAL IMPROVEMENT OF BIOCOMPATIBLE AND PHOTOCURABLE RESINS FOR 3D PRINTING FOR BONE REGENERATION USING ADVANCED MULTI-POROUS DESIGNS

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Keywords: Bone scaffolds; Polymeric biocompatible resins; TPMS structures; Bioactive materials.

Introduction and objective: Bone implants are widely employed to address increasing bone diseases involving significant tissue loss due to pathology evolution. However, the demand outpaces their limited availability. Tissue engineering (TE) is vital in creating biocompatible 3D-printed scaffolds using diverse materials [1]. These scaffolds must fulfill essential criteria, particularly biocompatibility, to promote cell proliferation, tissue adhesion, and proper gene signaling. The scaffold is a guiding framework for new tissue growth for bone tissue, necessitating high internal interconnectivity for vascularization, innervation, and nutrient transport. Mechanical strength is also critical to withstand bone tissue loads. This research aims to develop a resin fulfilling TE parameters for a biocompatible implant.

Methodology: Biocompatible and bioactive photocurable resin has been synthesized using the digital light processing (DLP) technique to create complex hierarchical structures. These resins are based on blends of polyethylene glycol diacrylate ($PEGDA_{575}$) and acrylic acid (AAc), using Irgacure 1173 as a photoinitiator. Additionally, nanohydroxyapatite (nHA) and β-tricalcium phosphate (β-TCP) are added to improve the biocompatibility, bioactivity, and mechanical resistance of the fabricated 3D pieces.

Results and discussion: With recent improvements in additive manufacturing (AM), the utilization of minimum triply periodic surface (TPMS) [2] structures is a viable approach for generating patient-specific components. These mathematical surfaces with zero local curvature are intended to diffuse the material's internal stresses, reducing the risk of local stress concentration (stress raisers), which might cause fractures and material failure. These TPMS structures replicate the porosities of bone tissue, reducing the requirement for porogen agents and allowing the integration of other bioactive compounds to improve biocompatibility and mechanical strength even further. Three different types of TPMS were tested in this study (primitive P, gyroid G, and diamond D) using both mechanical and biocompatibility tests. The printability was evaluated using FE-SEM and MicroCT. Finally, the chemical structure of the resins was measured using FT-IR spectroscopy.

Conclusions: The results indicate that the TPMS structures seem to considerably improve the mechanical resistance of the materials while maintaining the biocompatibility and bioactivity levels in a similar range compared to commercial resins, demonstrating that these resins are promising materials to be used as a base for bone scaffolds.

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STUDY OF *IN VITRO* DEGRADATION AND DRUG DELIVERY OF AN ENDONASAL MEDICAL DEVICE 3D PRINTED BASED IN PLDLA-TMC WITH MOMETASONE FUROATE

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Keywords: 3D printing; Drug delivery; PLDLA-TMC; Mometasone Furoate

Introduction and objective: The use of polymeric devices for drug delivery has gained significant importance in the scientific community. Loading mometasone furoate in these devices offers a promising alternative for the treatment of chronic rhinosinusitis. Additionally, the application of additive manufacturing in producing polymer scaffolds for medical purposes shows great potential in improving the quality of life for society. In line with this, a three-dimensionally printed endonasal device based on Poly(L-D,L-Lactic Acid – Trimethylene Carbonate) polymer (PLDLA-TMC), loaded with mometasone furoate, was developed. The selection of this polymer is based on its outstanding flexibility, excellent biocompatibility, and suitability for 3D printing application. The in vitro hydrolytic degradation of this device was evaluated, along with the assessment of the drug release profile of the printed device.

Methodology: The PLDLA-TMC polymer was utilized for loading mometasone furoate and subsequently used in three-dimensional printing to create a nasal device. Both the drug-loaded polymer and the printed device underwent FT-IR analysis for comprehensive chemical characterization. The printed device, without the drug, underwent an in vitro hydrolytic degradation test. At various degradation time points, the material was subjected to GPC, TG, DSC, FT-IR, and SEM tests to assess the physicochemical effects of the degradation process. Furthermore, the drug release profile of the printed material loaded with mometasone furoate was evaluated using PBS at 37 ^{o}C over a period of 30 days.

Results and discussion: The devices were printed using an Octopus® printer from 3DBS. To evaluate the hydrolytic degradation process of the polymer, an in vitro degradation assay was performed on the device without the drug. After 7, 14, 21, and 28 days, the material was assessed using GPC, FT-IR, DSC, TG, and SEM. The results did not indicate significant changes in the chemical and thermal properties of the polymer. However, GPC revealed a slight decrease in the weight and average molar masses of the polymer, suggesting degradation. Specifically, the weight and average molar masses decreased from 176 KDa and 340 KDa to 87 KDa and 190 KDa, respectively. Additionally, SEM showed the formation of micropores on the surface of the printed material.[1] The incorporation of mometasone furoate into the synthesized PLDLA-TMC took place via casting and was confirmed by the FT-IR assay, which displayed the main characteristic bands of both the drug and the polymer. The drug release profile of the device indicated a higher release in the initial hours (200 mcg/ml), with sustained release for 30 days (approximately 25 mcg/ml in the first 15 days and 10 mcg/ml in the last 15 days), suggesting that the drug was released throughout the experiment. This phenomenon could be attributed to the presence of micropores observed via SEM in the sample subjected to the drug release assay.[2]

Conclusions: The results indicate that the polymer used did not undergo significant degradation in the initial 30 days. However, this technology, developed from additive manufacturing, holds promising potential for the treatment of chronic rhinosinusitis. The device demonstrated sustained drug release over the course of 30 days, suggesting its viability for therapeutic applications.

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3D BIOPRINTING OF FIBROBLASTS FOR THE PRODUCTION OF QUANTIS® BIOIDENTICAL HUMAN COLLAGEN: AN INNOVATIVE APPROACH IN ADDITIVE MANUFACTURING

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Keywords: Collagen type I; 3D Bioprinting; Additive Manufacturing;

Introduction and objective: Emerging technologies in additive manufacturing allow remarkable advances in 3D printing of biocompatible materials, cells and complex structures[1]. Among the outstanding elements, type I collagen has played a key role in boosting the pharmaceutical, medical and cosmetic sectors[2]. In this context, given the growing trends in bioengineering and the search for ethical methods and designers, the present study aims at an innovative approach using bioprinting as a tool in the production of human collagen, offering a promising alternative to the use of animal sources.

Methodology: First, a 2D culture of human fibroblasts was carried out in free animal medium, followed by the production of a bioink composed of type 1 collagen, synthetic polymer, chemical factors and human fibroblasts. After a period of 4 days, the process of generation and decellularization and purification of the extracellular matrix produced in the construct was carried out (patented process). After that, analysis of SEM, SDS-Page, optical microscopy and quantification of collagen through the picro Sirius Red method of the constructs was carried out.

Results and discussion: After carrying out the entire process and adjustments that occurred during the course of the research, in the characterization analyzes (SEM and SDS-PAGE) it was possible to observe fibrillar material bioidentical to human collagen, with an integrated protein structure, and to recognize characteristic bands of each protein according to its molar mass, in particular the protein of collagen I, in addition, in the quantification of collagen, the production of 4 times more collagen was observed in the constructed ones when compared to 2D methods. Based on the high production of collagen in the three-dimensional environment, the building acts in this process as a cellular bioreactor, leading to high yields in the production of human collagen, with lower costs, using less space. the process of decellularization and matrix extraction, these cells are returned to the process and a new cycle begins.

Conclusions: Thus, with the process presented, it was integrated and dynamic proteins through the combination of new trends in bioengineering and 3D printing technology, thus hopeful for the development of a new era in collagen biomanufacturing, boosting applications in various industrial sectors, tissue engineering , regenerative medicine, for example, being used as dermal and joint fillers.

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DEVELOPMENT OF 3D PRINTER FILAMENT INCORPORATED WITH OXIDE FOR ORTHOSES

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Keywords: Filament, 3D printer, Oxide, antibacterial

Introduction and objective: Orthoses are crucial in rehabilitating individuals with physical limitations, providing mobility, and enhancing the quality of life. However, infections associated with medical devices remain a challenge [1]. In this case, the addition of oxide to PLA (polylactic acid) filaments was investigated, along with the supplementation of starch to reduce the Oxide's band gap [2]. This work focuses on producing PLA filaments augmented with oxide and starch through 3D printing technology. It aims to develop functional orthoses with enhanced antimicrobial properties with and without visible light.
Methodology: The nanocomposite filament was obtained in a single screw extruder (Wellzoom B2) at 90 °C, following a proportion of 89% PLA and 11% oxide and starch. In summary, the PLA/OXIDE/STARCH assembly was ground with balls and inserted into an extruder with a rotational speed of 30 rpm. The optical properties of the synthesized semiconductors were performed on a spectrophotometer in the UV–Vis region with diffuse reflectance accessory using Varian equipment (Cary 300). The antibacterial activity against the strain *Staphylococcus aureus* ATCC 25923 was evaluated, with the bacterial suspension on the blanket exposed to visible light (660nm, Laser DUO).
Results and discussion: The UV–Vis reflectance was used to determine the optical band gap energy (Eg) for óxide/starch. The estimated value for óxide/starch was 2,98eV. These values were lower than the value of Eg commonly reported for Oxide (3.37 eV)—the fundamental role of adding starch to the oxide, expanding its use to the visible spectrum. The direct contact test verified the antimicrobial activity of the filaments against S. aureus through the number of living cells present on the surface of the agar plates. It was observed that in the presence of light, there was an increase in the inhibition rate to 100%. Without the action of light, there was also a significant effect on the inhibition rate, with approximately 95%.
Conclusions: Using the UV-Vis reflectance technique, which found a bandgap reduction from 3.37 to 2.98eV, and the antibacterial test, which showed an efficiency of 95% in the absence of light and 100% in the presence of light, it can be stated that the use of filaments incorporated with Oxide and starch for the production of orthoses is extremely promising.

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TOPIC 2

Biomaterials characterization

GRAPHENE OXIDE REDUCTION BY DOPAMINE

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Keywords: graphene oxide; dopamine; reduction; process

Introduction and objective: Graphene-based composite materials, because of their high electrical and thermal conductivity, are attractive for applications in electronics, energy and medicine. Given their low cost and high performance, graphene oxide (GO) and reduced graphene oxide (rGO) are candidates for developing graphene/biopolymer composites. However, rGO synthesis uses strong oxidants, diminishing their conductive properties. Although using dopamine (DA) as a green reductor is an alternative, this process has not been sufficiently studied. Thus, this study aimed to evaluate the effect of the GO/DA ratio, temperature and reaction time in the GO/DA reduction process to determine an optimum.
Methodology: An experimental design 2^3 was used to evaluate the influence of variables GO/DA ratio (100:50- 100:75), temperature (60-70ºC) and reaction time (6-24 h) on the resulting data. The samples were analyzed qualitatively and quantitatively by FTIR, XRD, SEM and EDS techniques in addition to the reaction yield determination. The optimized responses were the yield, efficiency, atomic interspace and C/O ratio, which were evaluated using Statgraphics Centurion XIX software.
Results and discussion: The samples subjected to higher temperatures presented a lower yield due to the thermal elimination of the catechol groups present in the GO structure, which are released in the form of water due to the oxidation of DA [1]. Increasing the DA concentration in the solution produced high yields, which could be attributed to PDA autopolymerization [2]. A higher reaction temperature was necessary for shorter reaction times and higher yields.
From FT-IR spectroscopy analysis, all peak intensities corresponding to the oxygen-containing groups in the rGO samples decreased compared to those of the GO, indicating that they were partially eliminated. XRD analysis showed that the diffraction peaks shifted after the reduction due to oxygenated functional group removal. SEM images revealed morphological differences between GO and rGO samples. The elemental analysis confirmed the GO reduction, showing an increase in the C/O ratio. Thus, in order to get an effective DA/GO reduction, the reaction time should be 6 h, temperature should be 80ºC and GO/DA ratio should be 100:75.
Conclusions: The characterization of the material highlighted a successful reduction of GO by DA, where the reaction time, the temperature and GO:DA ratio were fundamental factors in the rGO synthesis.

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ULTRATHIN COLLAGEN AND GELATIN FIBERS: BENIGN SOLVENTS TO PRODUCE POTENTIAL BIOMIMETIC BIOMATERIALS BY SOLUTION BLOW SPINNING

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Keywords: collagen; gelatin; biomaterials; solution blow spinning

Introduction and objective: The main constituent of extracellular matrix (ECM) of various tissues is collagen, a biodegradable and biocompatible protein with excellent regenerative properties. A promising way to produce scaffolds, artificial ECM, involves the production of nanometric or submicrometric fibers, dimension of the natural fibers found in the ECM of many tissues. Solution blow spinning (SB-Spinning) allows the production of fibers with high feed rates and *in situ* deposition. Here we produce ultrathin collagen and gelatin (polymer obtained from collagen denaturation) fibers by SB-Spinning using solvents that preserve the integrity of the polymers and evaluate the morphology and properties of these fibers [1,2].

Methodology: Collagen (10 wt%) and gelatin (10 and 15 wt%) solutions were prepared in 90 wt% acetic acid under stirring overnight at room temperature. A glass syringe was used to spin 25 cm (for gelatin) or 20 cm (for collagen) away from the collector using 30 psi (gelatin) or 10 psi (collagen) of pressure at 3.6 to 10.8 mL/h for gelatin and at 3 to 6 mL/h for collagen. The fibers were characterized by scanning electron microscopy, differential scanning calorimetry and polyacrylamide gel electrophoresis.

Results and discussion: Gelatin and collagen submicrometric fibers were produced from 90% acetic acid solutions, a benign solvent that allows the solubilization of high amounts of these polymers and present low toxicity with low cost, what reveals it as a promising benign solvent for this application. Gelatin fibers presented average diameters between 740 ± 299 nm and 909 ± 326 nm for 15% solutions and between 175 ± 64 nm and 196 ± 113 nm for 10% solutions, indicating the direct effect of polymer concentration on the diameter of fibers. Collagen fibers presented average diameters between 542 ± 185 nm and 543 ± 242 nm, being thicker than the gelatin fibers obtained with the same polymer concentration, an indicative of the preservation of the natural structure of these protein, the triple helix secondary structure. The preservation of collagen triple helix after the spinning process was confirmed by differential scanning calorimetry and gel electrophoresis results.

Conclusions: Aqueous acetic acid was considered a good solvent for the solubilization of these collagenous proteins, being adequate to the production of biomaterials due to its low toxicity and ability to preserve the natural structure of collagen. It was also possible to understand the effect of some production parameters on the diameter of these fibers, an important step in the development of new biomimetic biomaterials produced by solution blow spinning.

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STUDY OF POWDER METALLURGY TECHNIQUE FOR OBTAINING Mg-Zn SYSTEM ALLOYS FOR BIOMEDICAL APPLICATIONS

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Keywords: Mg-Zn; dry high-energy milling; natural pores; biomaterials.

Introduction and objective: The present study aimed to investigate the powder metallurgy technique for obtaining Mg-Zn systems with potential for biomedical applications [1]. The use of magnesium alloys has gained prominence in the biomaterials field due to their mechanical properties similar to bone. The objective of this study was to evaluate the efficiency of the dry, high-energy milling technique in obtaining Mg-Zn alloys with improved mechanical properties and porosity.

Methodology: The powder metallurgy technique was used to produce Mg-Zn systems using dry, high-energy milling in a SPEX 8000D mill [2]. Samples were obtained using high-purity Mg and Zn powders in defined proportions. The powders were dry milled using tungsten carbide jars and balls, with varying milling times, in a controlled environment with argon atmosphere. The characterization of the alloys was performed by X-ray diffraction analysis and scanning electron microscopy.

Results and discussion: The results showed that the dry, high-energy milling technique was efficient in producing Mg-Zn alloys with reduced and homogeneous particle sizes. X-ray diffraction analysis confirmed the formation of a solid phase with hexagonal structure, low contaminant concentrations, and absence of amorphous regions. Scanning electron microscopy revealed a refined and homogeneous microstructure with a flocular morphology, which provided natural pores in the shaped structure.

Conclusions: Thus, the study indicates that high-energy milling is a promising alternative for obtaining Mg-Zn systems with improved morphological characteristics. The obtained systems showed a refined and homogeneous microstructure with reduced particle sizes and flocular morphology, contributing to increased porosity and osseointegration. Therefore, the powder metallurgy technique, using high-energy milling, can be considered an interesting alternative to produce Mg-Zn systems with potential for biomedical applications.

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NEW BIOMATERIAL BASED ON DECELLULARIZED EXTRACELLULAR MATRIX FUNCTIONALIZED WITH NANOCHITOSAN TO ELABORATE BIODRESSING

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Keywords: Tissue engineering; Biodressing; Decellularized extracellular matrix; Nanochitosan.

Introduction: The skin is an organ that has great reparative capacity, but some acute and chronic injuries can trigger significant loss of its architecture. In these cases, tissue engineering can be applied through the use of scaffolds [1]. Biomaterials composed of decellularized extracellular matrix (dECM) show low immunogenic response and provide a favorable environment for cell adhesion and proliferation. Natural polymers such as nano chitosan have been gaining prominence in biomedical applications for their healing and antimicrobial properties [2]. **Objective:** This work proposes the development of a dECM biomaterial functionalized with nanochitosan for use as a biodressing.
Methodology: To obtain dECM, bovine skin fragments were decellularized by stirring in 1% SDS medium, EDTA, Aprotinin and ATB. The nano chitosan was obtained from the ionic gelation method, and characterized by DLS, zeta potential, MET, SEM, EDS and antibacterial activity. The decellularized fragments were functionalized with the nanoparticles by adsorption. The characterization of the scaffold was done by histological analysis with H&E, Gomori Trichrome and Alcian Blue staining, DNA extraction, immunofluorescence, SEM, EDS and FTIR.
Results: The hydrodynamic radius of chitosan nanostructures ranged from 75.48 ± 13.73nm, and polydispersity index of 0.393 ± 0.015. The antimicrobial activity of nano-chitosan was observed at all tested concentrations (0.26 µg/ml-1 to 19.7 µg/ml-1) for *Escherichia coli, Enterobacter cloacae, Salmonella sp.* and *Staphylococus aureus bacteria*. The efficacy of the matrix decellularization process was proven by DNA extraction, with no characteristic bands in the decellularized sample compared to the control. Immunofluorescence showed the absence of cell nuclei in the decellularized tissue, leaving only some debris. The absence of nuclei was also visualized by histological analyses, in which it was possible to observe the maintenance of a large part of the matrix structure through the qualitative evaluation of collagen fibers and preservation of glycosaminoglycans. FTIR showed the presence of regions of possible intermolecular interactions between the matrix and the nano-chitosan. The material showed good adsorption of the nano chitosan in the matrix (50.4%). SEM analysis showed that the dECM scaffold has adequate structure and porosity to favor cell adhesion and growth.
Conclusion: Thus, this work generates perspectives of nanotechnological biodressing from xenogeneic sources for tissue repair.

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HOMOGENISATION OF TITANIUM SCAFFOLDS USING MicroCT AND FEM

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Keywords: Scaffolds; Homogenisation; MicroCT; Elastic Properties

Introduction and objective: Severe bone fractures often require internal fixations for treatment. In unhealthy patients, post-implantation bone fractures can occur because mismatches in stiffness between the bone and the implant biomaterial. To alleviate this, the biomaterial microstructure can be designed to mimic the effective stiffness of bone [1,2]. In the case of 3D printed titanium scaffolds, the prediction of the lattice's effective properties using FEM is not straightforward due to reported numerical results do not agree with experimental data [2]. Here, a comparison of the effective stiffness between microCT-FEM models and experimental data was performed to evaluate the impact of geometrical irregularities.
Methodology: A microtomography (microCT) was performed over Direct Selective Laser Melting (DSLM)/Ti-6Al-4V ELI cubic scaffolds using a Nikon XT H 160 system. A 360° scanning was applied over the samples using steps of 0.36°, resulting in voxels of 0.28 µm. The microCT data was converted to finite elements method (FEM) models taking special care in keeping the geometrical irregularities. Titanium Young's modulus and Poisson ratio were considered as 110 GPa and 0.3, respectively. However, since titanium properties depend on the size of the rods, other values were analysed. Effective elastostatic properties were evaluated using the average 48haracterizati technique [1].
Results and discussion: The resolution of the microCT successfully captured the geometrical irregularities, like attached powder particles. To capture such details on the FEM model, the mesh of the unitary cell of the lattice reached 1.3 million of linear tetrahedral elements. Results show that the Young's modulus and Poisson ratio of the lattice are 12.54Gpa and 0.307, respectively. Results agreed with numerical models reported in the literature but differ from experimental data [2]. These differences, also observed by literature, were previously attributed to model simplifications that remove the geometrical irregularities. Here, it was shown the irregularities do not significantly account for the gap between the numerical end experimental results. Since the stiffness of 3D printed titanium decrease depending on the characteristic length of the scaffold rods, other stiffness titanium values were explored resulting in more seamless effective properties. However, no total matches between experimental and numerical results were achieve.
Conclusions: The microCT-FEM model results show that irregularities are not the main reason of the differences between numerical and experimental data. Since the only two factors that affect the average 48haracterizati technique are the geometry and the elastic properties, and taking into account that properties depend on the characteristic length, it was conclude that the elastic properties of the microscale rod titanium may have a different value compared to the values reported for macroscale samples.

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PRODUCTION OF POLYCAPROLACTONE/CURCUMIN MEMBRANES AND ITS EFFECTIVENESS IN CELLS GROWTH, pH SENSITIVITY AND RELEASE OF CURCUMIN

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Keywords: nanofibers; biomaterial; curcumin; polycaprolactone

Introduction and objective: The development of advanced wound dressings (AWD) has been increasingly widespread in recent years. PCL is a biodegradable polymer widely used to produce biomaterials for drug delivery. Extracted from *Curcuma longa* rhizomes, curcumin (CC) is a polyphenol with antimicrobial, anti-inflammatory and pH indicator properties. The present study discusses the production of polycaprolactone (PCL) mats incorporated with CC by Solution Blow Spinning (SBS), a technique that allows the production of ultrafine fibers. The fibers were analyzed by scanning electron microscopy (SEM) and the membranes were characterized regarding their *in vitro* cytotoxicity, sensitivity to pH, solubility and CC release in water. [1,2].

Methodology: An 18 wt% PCL solution in chloroform/acetone (3:1) was prepared under stirring for 2h at 60°C. PCL/CC solutions were prepared by adding 3 wt% of CC (in relation to polymer mass) to PCL solution. A glass syringe was used to spin 25 cm away from the collector using 25 psi of pressure at 30 mL/h. L929 cells were used to evaluate the membranes cytotoxicity, Fourier-transform infrared spectroscopy (FT-IR) was used to evaluate the interactions of their components while the diameter and morphology of fibers were characterized by SEM. CC release, pH sensitivity and solubility tests were also performed.

Results and discussion: The PCL mats presented a white color while the PCL/CC mats resulted in a bright yellow color that turns orange and reddish with the increase in the pH value of tested solutions. Although some color changes were observed with pH increase, in the pH range observed in wounds (between 4.0 and 9.0) was not evident the color changes, not allowing the direct visual observation of pH changes in wounds, since the more perceivable color changes are observed above pH 9.0. The ultrafine fibers of the mats were characterized by SEM, allowing the observation of their morphology and the interactions observed between curcumin and PCL were elucidated by FT-IR. The water solubility value observed after 72h was 11.66 ug/mL for PCL/CC membranes and PCL membrane did not solubilize. Despite the hydrophobic nature of curcumin, this bioactive compound was released in water, what indicates the ability of the PCL/CC membranes to deliver this potential antimicrobial molecule. The cytotoxicity test, performed with mouse fibroblast cells resulted in 92.3±7.4% and 116.3±11.1% of viable cells for PCL and PCL/CC membranes, respectively. Since AWD are directly involved in fibroblast cells growth during wound repair, this result indicates both mats as promising candidates to produce AWD.

Conclusions: Here, the effectiveness of PCL/CC membranes releasing curcumin was confirmed. In addition to the release of CC even in water, it was observed that cells growth was greater in PCL/CC membranes than in PCL membranes. Changing color according to pH sensitivity did not prove to be advantageous, since there is no perceptible change in the targeted pH ranges. The morphology of the ultrafine fibers and interactions of the polymer with the CC were adequate for the purpose of the dressing.

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ASSESSMENT OF CORROSION RESISTANCE ON THE SURFACE OF NANOTEXTURED STAINLESS STEEL COATED WITH A THIN COPPER FILM FOR BIOMEDICAL APPLICATION

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Keywords: Hierarchical Surfaces, Corrosion Resistance, SS 316LVM

Introduction and objective: 316 LVM stainless steel is used as raw material for manufacturing medical devices and implants. Contamination of these devices with bacteria is an important factor to be observed, as the formation of bacterial biofilm can cause the loosening and loss of the device. To minimize this problem, the surface of 316 LVM steel has been modified to promote nanotexturing of its surface. Thus, the objective of this research is to evaluate the corrosive behavior of these surfaces, since these devices, when inserted into the human body, can trigger inflammatory reactions from corrosion products of the material [1].
Methodology: 316LVM stainless steel samples were cut into T-profile, sanded (SiC #220), cleaned with acetone in an ultrasonic bath, dried, and stored. They were then subjected to cathodic plasma electrolytic oxidation (CPEO) treatment, followed by corrosion under an H2SiF6 solution and subsequent deposition of a thin copper film. Following, samples were subjected to open circuit potential and potentiodynamic polarization tests. This system comprises a potentiostat Ivium, with a saturated calomel reference electrode and the working electrode being the treated and untreated 316LVM samples with an exposed area of 36 mm^2, using SBF solution as the electrolyte.
Results and discussion: Samples modified by the combined treatment of CPEO, acid corrosion, and deposition of a thin copper film (316LVM-NT) showed better performance in terms of their corrosion resistance when compared to the untreated 316LVM samples and those subjected to the sanding process (316LVM-L). These results are likely due to the layer of iron oxide obtained during the CPEO process, which provides the material with greater corrosion resistance.
Conclusions: The combination of treatments applied to the surfaces of 316LVM stainless steel made it possible to improve the material's corrosion resistance when compared to the untreated surface. It is believed that the main factor for the increased corrosion resistance of 316LVM-NT occurred due to the presence of oxide phases on the material surface, which was formed during the CPEO process.

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STUDY OF HIGH-ENERGY MILLING AND SINTERING OF MG-ZN SYSTEM ALLOYS

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Keywords: Mg-Zn; high-energy milling; sintering process ; biomaterials.

Introduction and objective: The Mg-Zn system is a promising alternative for biomedical applications due to its mechanical properties and potential biocompatibility. This study aims to investigate the effect of high-energy milling on the microstructure and properties of Mg-Zn alloys, as well as to evaluate the influence of the sintering process on the mechanical properties of the samples. The goal is to obtain alloys with better mechanical performance for possible biomedical applications [1,2].

Methodology: The Mg-Zn system alloys were prepared by high-energy milling and sintering. High-energy milling was performed using a SPEX 8000D high-energy mill, with tungsten carbide balls and jars [2]. The samples were compacted using a hydraulic press and sintered in a controlled argon atmosphere. The samples were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), and compression mechanical tests.

Results and discussion: The microstructure of Mg-Zn alloys was significantly refined by high-energy milling, resulting in a decrease in the average grain size and a floc-like morphology. Sintering showed a significant effect on the mechanical properties of the alloys. Additionally, increasing the Zn content in the alloys resulted in an increase in compressive strength and hardness. The results suggest that high-energy milling and sintering are effective methods for improving the mechanical properties of Mg-Zn systems.

Conclusions: This study demonstrated that high-energy milling and sintering are effective in obtaining Mg-Zn systems with improved mechanical properties. Sintering significantly affects the mechanical properties of the samples. Increasing the Zn content in the alloys increases the tensile strength and hardness. The results obtained are relevant for the development of new biomaterials with better mechanical properties for biomedical applications.

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EFFECT OF THE MEDIUM ACIDITY ON THE CORROSION RESISTANCE OF Ti-10Mo-30Nb ALLOY FOR BIOMEDICAL APPLICATIONS

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Keywords: Biomaterials; Titanium alloys; Corrosion; microstructure.

Introduction and objective: β-Metastable Ti alloys are attractive materials for dental and orthopaedic implants due to their good biocompatibility, biocorrosion resistance and mechanical properties, including low Young's modulus. Previous studies had shown that the treated Ti-10-Mo-30Nb alloy presented good properties, indicating a great potential for biomedical application. However additional studies are needed such as corrosion resistance. Therefore, the objective of this work was to analyse effect of the medium acidity on the corrosion resistance of Ti-10Mo-30Nb treated at 950 °C/1h and then water quenched.
Methodology: The electrochemical behaviour was performed in a cell of three electrodes: working electrode -titanium alloy, reference electrode - saturated calomel and against electrode - platinum wire. The corrosive medium consisted of a solution containing chloride ions, naturally aerated at pH 1 and pH 5.5. All assays were performed at room temperature. The corrosion resistance of the alloy was evaluated by monitoring the open circuit potential, polarization curves and cronoamrometry.
Results and discussion: The results showed that in relation to the open circuit potential, significant differences were observed between the Ti-10Mo-30Nb and Ti-6Al-4V alloys. The alloy presented higher corrosion resistance than Ti-6Al-4V alloy.
Conclusions: Ti-10Mo-30Nb alloy showed to be more resistant to corrosion when compared to the commercially Ti-6Al-4V alloy.

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DEVELOPMENT OF MICROFIBRILLAR MEMBRANES BASED ON POLY(E-CAPROLACTONE) TO BE USED IN THE TREATMENT OF CUTANEOUS LESIONS

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Keywords: Polymeric biomaterials; rotary jet spinning; wound dressing.

Introduction and objective: Using skin dressings becomes essential to help the complex healing process and prevent external threats. A wide range of materials can be used to develop dressings. However, polymeric materials, such as poly(ε-caprolactone)/PCL, and hydrogels, such as alginate, stand out. PCL has interesting properties as biocompatibility, non-toxicity, and good mechanical properties. Alginate is a hydrophilic, biocompatible, and biodegradable hydrogel. Furthermore, these characteristics can still be associated with bioactive ingredients, such as Pracaxi oil, preventing local infections. Therefore, the objective was to obtain PCL membranes, modified with calcium alginate hydrogel and impregnated with Pracaxi oil, to be used as a wound dressing.
Methodology: PCL (20% m/v) was obtained from the rotary jet spinning technique at room temperature to act as a base layer for the groups: PCL-0, PCL - alginate (PCLA-2%, PCLA-4%, PCLA-6%) and PCL - alginate – Pracaxi oil (PCLOA-2%, PCLOA-4% and PCLOA-6%). The materials were characterized in terms of composition in fatty acids of Pracaxi oil via gas chromatography (GC), morphology (scanning electron microscopy/SEM) and crystallinity (x-ray diffraction/DRX).
Results and discussion: In gas chromatography (GC), the presence of saturated and unsaturated fatty acids was identified, with oleic, linoleic, and behenic acids as the majority. These acids contribute to the healing process [1]. The morphological characterization of the material (SEM) demonstrated the presence of several pores in the PCL-0 sample (diameters ranging from 18.40 to 19.50 µm); however, in the PCLA-2% samples, the pores were covered by the alginate hydrogel. On the other hand, in the PCLA-4%, PCLOA- 2% and PCLOA-4% samples, it was possible to verify the presence of two different surfaces, characterizing a bilayer-type multizonal membrane. Discontinuous compositions and transition gradients of the materials were observed. The X-ray diffraction results showed a semi-crystalline structure.
Conclusions: It's concluded that the rotary jet spinning process, linked to the use of a volatile solvent, successfully contributed to the formation of microfibrillar PCL membranes with high porosity, indicating a promising potential for cell adhesion, proliferation, and increased hydrophilicity of material. The samples from the PCLOA group, with a multizonal profile, were selected as good potential candidates for accelerating the healing process due to the presence of bioactive compound and structural composition.

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STUDY OF THE CORROSION PROPERTIES IN FUNCTION NB ADDITION IN THE Ti-10Mo-xNb ALLOYS FOR BIOMEDICAL IMPLANTS

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Keywords: Metallic biomaterials; Ti alloys; Microstructure; Corrosion.

Introduction and objective: New titanium alloys have been developed for biomedical application, for example in the manufacture of orthopedic and orthodontic implants. This effort is mainly due to some specific properties that Ti beta alloys have, such as mechanical strength, biocompatibility, and corrosion resistance. Different beta stabilizing alloying elements are used in the formulation of these alloys such as Mo, Nb and Zr whose combination controls the present phases, the mechanical resistance and the Young's modulus. The aim of this work is to present a systematic study on the effect of increasing Nb addition on the corrosion resistance of a Ti-10%Mo titanium alloy.

Methodology: The evaluation of the electrochemical behavior of Ti-10-Mo-xNb $0 \leq x \leq 30$ alloys was carried out by monitoring the open circuit potential and potentiodynamic polarization curves. The tests were obtained using a physiological solution of 0.9% NaCl at room temperature. The analyzes were carried out in a three-electrode cell: saturated calomel was the reference electrode, platinum as the counter electrode and the TiNb alloys as the working electrode. The open circuit potential (OCP) was monitored for 1 hour. The polarization curves were obtained in the potential range -0.25 to 1.60 V with a potential scan rate 0.001V/s.

Results and discussion: The results showed that in relation to the open circuit potential, no significant differences were observed between the different analyzed alloys. Regarding the polarization curves, the presence of two distinct regions: the first one, where the current density increases with the potential, the second region starts around -0.15 V, where it is observed the presence of a passive region. The presence of niobium in the alloys promoted a reduction in the current density in both regions. Although there was no significant difference in corrosion resistance in the anodic region of the curves as a function of the different niobium concentrations **Conclusions:** The results showed that the increasing addition of niobium promotes the formation of more corrosion resistant films.

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CHARACTERIZATION OF CRUDE CORN ZEIN MICROSPHERES OBTAINED BY ANTI-SOLVENT-DIALYSIS METHOD WITH/WITHOUT THE AID OF ULTRASOUND

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Keywords: corn zein; microspheres; ultrasound, biomaterials

Introduction and objective: Corn zein is a protein of the prolamines classes of cereals present about 8%(w/w) in the endosperms of corn kernels. Zein consists of large amounts of hydrophobic amino acids, which makes it insoluble in aqueous, soluble only in hydroethanolic medium(70-95%), and other polar organic solvents, such as, methanol, isopropanol and acetone, but ethanol is easier to find[1]. In addition, the advantage of this process is that in the same extraction solvent of zein can use to make the microspheres, so eliminating the subsequent steps to form particles, optimizing time and spending of energy for purification.
Methodology: 25cm of cellulose dialysis tubes (Viskase) were activated in 1L of deionized water for 4h at 25◦C, before dialysis. 5g crude zein (purchased from Wilson Oshiro) were dissolved in 100mL (75%EtOH), placed in this tubes (16kD) and sealed on both sides to prevent spillage, then was placed in the dialyzer containing 1L of deionized water as antisolvent with/without ultrasonic bath for 30min. The dialysis system was left in continuous magnetic stirring for 36h at 25◦C. After this time, the dialysis contents were obtained and lyophilized. White zein powder was stored at room temperature for future characterization and compared with Aldrich zein. The microspheres size and morphology were analyzed by scanning electronic microscopy (SEM) after sputtering with gold.
Results and discussion: After dialysis, there was the formation of a whitish substance at the bottom of the dialysis tube (purified zein) and at the top the presence of carotenoids (yellow-orange color). They presented a very large amount of zein still present in these carotenoids giving a low yield of purified zein in the form of spheres, but it may be possible to perform the purification using a system of reverse osmosis in nanomembranes. This system that are used for desalination of seawater or brackish, but for hydroethanolic medium, which will be carried out in the future on a pilot plant scale. The crude zein concentration at 5.0% (w/v) in hydroethanolic solutions at 75%, has the whiter zein than 10% after dialysis and Aldrich's zein. The SEM photomicrographs show that the zein microspheres obtained with 5% (m/v) crude zein are uniform, free and smooth than those obtained with 10%, because the latter contained more carotenoids agglutinated in the microspheres. Any nanospheres were'nt seen in any photomicrographs, because they agglutinated with carotenoids. Millimeter spheres of zein with nanoparamagnetic magnetite are very interesting to obtain immobilized amylases to produce ethanol from starch.
Conclusions: Antisolvent dialysis may be the future purification method with a similar process on an industrial scale, type reverse osmosis membranes compatible with hydroethanolic solutions. Not only get the purification, but also have an extraction process to reduce the price of zein, even if it's raw (80% purity) at a level of about U$3.00 – 4.00/kg and not U$300.00 from Aldrich[2]. This method to produce easily microspheres maybe also nanospheres to delivery drugs or immobilized enzymes to recovery and reuse. This work follows the premise of the SDG 9.

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SANDWICH-LIKE FILM FOR ON-DEMAND TOPICAL RELEASE OF LIDOCAINE

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Keywords: Sandwich-like film; on-demand release; lidocaine; transdermal drug delivery.

Introduction and objective: Transdermal drug delivery has many advantages over conventional administration, including avoiding the first-pass effect in the liver, reduced side effects, and improved patient compliance [1]. An increase in body temperature increases the blood flow on the skin, which can play a significant role in transdermal drug delivery. Furthermore, the average body temperature is about 37 °C, but various conditions and illnesses can raise body temperature. As the blood flow and drug distribution are related, a mild rising temperature (37 °C to 45 °C) can be used to increase drug circulation and therapeutic outcomes [2].

Methodology: Briefly, the sandwich-like films were prepared using the casting technique. The top and bottom layers comprised c gum, whereas the middle layer comprised Eudragit® S100, PCL-T (300 g mol^{-1}), and lidocaine. The sandwich-like films containing (SL@LID) or not (SL) lidocaine were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) spectroscopy, and contact angle (OCA). Finally, the mechanical properties and the lidocaine release triggered by temperature were also investigated.

Results and discussion: The SEM images revealed that the films showed a sandwich-like structure, in which the top and bottom layers were comprised of PVA, glycerol, and xanthan gum, and the middle layer was comprised of Eudragit® S100 and PCL-T (300 g mol^{-1}). The SL and S-L@LID films showed elastic modulus values of 2.72 ±0.006 MPa and 2.70 ± 0.007 MPa; maximum strain values were 9.39 ± 0.087% and 9.71 ± 0.117%, respectively. As expected, the bottom and top layers showed similar contact angles (~56°), whereas the middle layer showed the highest contact angle value (~70°) mostly due to the hydrophobic nature of Eudragit® S100, PCL-T, and lidocaine. The FTIR spectra revealed collectively that lidocaine was successfully incorporated into the sandwich- like film. The release profile of lidocaine, an anesthetic with low solubility in water (9 $mg.mL^{-1}$), from the SL@LID film was evaluated at temperatures of ~37°C and ~42°C, which mimic body temperature under normal conditions and inflamed/infected, respectively. Overall, the amount of lidocaine released from the SL@LID film was greater at ~42°C (~100% within 80 min) than at ~37°C (~10% within 80 min), suggesting that the lidocaine release from the sandwich-like film prepared can be triggered by changing the temperature.

Conclusions: In summary, in this study, the sandwich-like film prepared was able to modulate the lidocaine release by changing the temperature, showing its potential use for on-demand topical release.

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LIPASE-RESPONSIVE NANOPARTICLES FOR ANTIBIOTIC DRUG DELIVERY

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Keywords: Lipase-responsive release; sulfamethoxazole; polycaprolactone-triol; nanoparticles.

Introduction and objective: Antibiotics release from the drug delivery system can be controlled by exploiting the microenvironment of bacteria (e.g., toxin production, pH, and temperature variations) as a stimulus to initiate a bacterial-triggered release. Such triggered release provides the benefits of on-demand delivery and reduces unnecessary exposure to antibiotics, thereby reducing the potential risk of resistance. Bacterial virulence factors (including lipases) are secreted by bacteria in abundance to elicit host damage, exert dominance and increase the survival rate of bacteria [1].

Methodology: The PCL-T-based nanoparticles loaded with sulfamethoxazole were prepared using the oil-in-water emulsion solvent evaporation method. The nanoparticles were characterized by dynamic light scattering (DLS), zeta potential, and the release conducted in the absence and presence of *Pseudomonas* lipase. The release of sulfamethoxazole from the PCL-T-based nanoparticle under the function of lipase was investigated using dialysis bags, in which 0.25 mL of PCL-T-based nanoparticles loaded with sulfamethoxazole (concentration of 0.25 or 0.50 $mg.ml^{-1}$) were diluted at 0.25 mL of phosphate-buffered solution pH 7.2 containing (at a final concentration of 0.42 $mg.mL^{-1}$) or not (control) *Pseudomonas* lipase.

Results and discussion: The PCL-T-based nanoparticles pure and loaded with 0.25 $mg.mL^{-1}$ and 0.50 $mg.mL^{-1}$ of sulfamethoxazole showed diameter sizes of 750.88 (0.036), 547.39 (0.006) and 922.39 (0.247) nm, respectively. The zeta potential values ranged from -38.37 mV to -8,51 mV. Lipase degrades PCL-T by hydrolysing the ester bonds in the polymer chain. In the presence of the enzymes, PCL-T-based nanoparticles showed a rapid release of sulfamethoxazole followed by a gradual and continual release over time. In comparison, PCL-T-based nanoparticles showed a significantly lower release of sulfamethoxazole in the absence of lipase. The results confirm our hypothesis that PCL-T-based nanoparticles degrade slowly in an aqueous medium due to the hydrophobicity, but the presence of lipase can induce the rapid degradation of PCL-T. The findings so far confirm that we have generated an enzyme-responsive nanoparticle system that can be further examined in bacterial cultures.

Conclusions: In summary, in this study, the PCL-T-based nanoparticles were able to modulate the release of sulfamethoxazole in the presence of a bacterial stimulus, such as lipase.

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CHARACTERIZATION OF CHITOSAN HYDROGEL FOR OSTEOARTHRITIS TREATMENT

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Keywords: cartilage; chitosan; hydrogel; osteoarthritis.

Introduction and objective: Osteoarthritis, a joint disease characterized by inflammation, pain and functional limitations, represents a significant clinical challenge. In this context, hydrophilic polymer hydrogels have emerged as a promising therapeutic approach. These hydrogels have properties similar to those of the cartilage extracellular matrix (ECM), making them ideal candidates. Among the polymers used, chitosan polysaccharides stand out, which have a chemical structure similar to that of glycosaminoglycans, proteins abundant in cartilage. In this context, this study aimed to characterize the physicochemical properties of hydrogel, aiming at the development of therapeutic interventions for the treatment of osteoarthritis.
Methodology: The hydrogel was manufactured by dissolving 2.5% w/v chitosan in 0.1 M HCl; urease dissolved in phosphate buffered saline (PBS) was added at concentrations of 25 U/mL or 50 U/mL and 7.5 or 10 M urea dissolved in Milli-Q water. The pH was evaluated at specific time intervals. The gelation test consisted of pouring the hydrogel every 30 seconds until it did not flow, recorded as gelation time. In the degradation test, the hydrogel was weighed and placed in tubes with 5 mL of PBS at pH 7.4 during the experimental times, and then weighed again.
Results and discussion: The results demonstrated that chitosan hydrogels from the 50U/mL urease groups exhibited a shorter gelation time compared to the 25U/mL urease groups, with times of 15 and 30 minutes, respectively. Therefore, the gelation test indicated that higher urease concentrations led to a decreased gelation time. Additionally, a pH increase over time was observed in all groups, with the 50U/mL urease and 10 M urea group showing a faster pH rise than the others. These findings support the study by Chenite *et al.* [1] which reveals that chitosan hydrogels form gels as their pH transitions from acidic to neutral, and shorter reaction times are associated with higher urease concentrations. In the degradation test, all groups experienced a decrease in mass, with the 5-day period exhibiting a lower degradation rate compared to the 1- day period. This decrease can be attributed to a reduction in crosslinking density, leading to an increase in mass due to swelling [2]. The 50U/mL urease group displayed a lower degradation rate compared to the 25U/mL urease group at 1, 5, 10, and 15 days. Aside from the clinical benefits, this approach holds the potential for significant positive economic and social impacts on society.
Conclusions: The results suggest that the chitosan hydrogel 50 U/mL urease and 10 M urea is the best candidate for further studies, as it showed a safe gelation time and pH stability for *in vivo* applications, in addition to its slower degradation that allows for longer effects on the osteoarthritic joint.

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PREFILLED SYRINGES WITH SILICONIZED SURFACES: AN OVERVIEW

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Keywords: lubricant; medication; silicon; syringes;

Introduction and objective: In Brazil, after creation of ANVISA and the establishment of minimum criteria for the distribution of biomaterials for medical and hospital purposes, there was a large increase in cases of complaints of quality deviations in that biomaterials, noticed by end users and/or health professionals. Therefore, the objective of the present work is to carry out a systematic review of these quality deviations related to excess lubricant in syringes since the year 2007, until the year 2022, and study how the lubricant present in the syringe interface could compromise the dosage of medication when they are in contact with certain drugs.

Methodology: The data collected on the lubricant presented in Notivisa refer to a visual evaluation, which does not mean that the amount of lubricant is outside the maximum limit required by the standard (0.25mg.cm-2), but configures that the visible excess can cause some alteration when coming into contact with the medicine, such as leaving the solution cloudy or whitish, for example. Following this principle, the results will be compared with results in the literature that show whether this lubricant may be interacting with certain medications.

Results and discussion: The government regulatory action by ANVISA is carried out through monitoring and publishing a spreadsheet, known as Notivisa. With respect to syringe, it is permitted lubricant according to the pharmacopoeia and ISO 7886-1:2017 (sterile hypodermic syringes for single use) and ISO 8537:2016 (sterile single-use syringes, with or without needle, for insulin). The lubricant is used to reduce friction, helping in the movement of the piston in the cylinder, but the excess of silicone present in the syringe may pose a risks to human health. The articles used as the basis for this review will be of paramount importance for the discussion of the results, that will serve as the basis for evaluating the results and how or if silicone can compromise some medications. Therefore, the results presented will be grouped according to each year, since 2007, when ANVISA determined the compulsory certification of these biomaterials, in order to make a chronology if this data collection has shown a trend. Then, the data will be evaluated for transportation, packaging and handling. Then, the results will be compared with those obtained in the literature regarding the interaction of this lubricant with medications and how it can be presented visually.

Conclusions: Syringes are biomaterials widely used for medical purposes, however your inspection is carried out on small samples of large batches. Therefore, a large number of irregularities may arise that were not identified at production process. Thus, this work aims to present failures related to the excess of lubricant noticed by the final consumers, that have potential to contaminate the medication and it may also be linked to interfering with the dosage of active ingredients and/or changing some aspect of solutions.

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IN VITRO MINERALIZATION OF β-TRICALCIUM PHOSPHATE AND BIOACTIVE GLASS CONSOLIDATED BY COLD SINTERING PROCESSING

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Keywords: Cold sintering processing, densification, bioceramics, S53P4 bioactive glass, β-tricalcium phosphate

Introduction and objective: Cold sintering processing (CSP) is a new consolidation technique that has shown to be a promising alternative to the traditional sintering on densifying ceramic bodies. CSP is primarily reported for electronic, mechanical and refractory applications. Recently, it has started to arise interest on the densification of ceramics applied on bone regeneration. However, few bioceramics are reported on it and the use of CSP on producing ceramic composites is still limited. Thus, this work aims to produce β-tricalcium phosphate/ bioactive glass (β-TCP/BG) pellets consolidated by CSP, comparing its physical and chemical properties with pellets consolidated by traditional sintering (TS).
Methodology: To produce CSP pellets, β-TCP (70 wt.%) and S53P4 BG (30 wt.%) powders were mixed in an agate mortar together with 10 wt.% of water. The mixture was added to a stainless-steel matrix with inner diameter of 15 mm. The matrix was heated to 200 °C while applying 166 MPa of pressure, with 30 min of dwelling time. TS pellets were produced by pressing the dry powder mixture and sintering them at 1200 °C. The pellets were characterized by scanning electric microscopy (SEM), X-ray diffraction (XRD), regarding their bulk density by the Archimedes method and their mineralization in simulated body fluid (SBF).
Results and discussion: Pellets consolidated by CSP method presented higher densification than pellets consolidated by TS, with bulk density of 82 ± 5% and 72 ± 1%, respectively. The value obtained to CSP pellets is similar to the reported when sintering pure 45S5 BG by cold isostatic pressing (CIP), of 75% by Nawaz et al [1] and 98% by Taveri et al. [2]. The SEM images revealed the presence of a highly densified surface on both CSP and TS-pellets, which corroborates with the bulk density results. While TS-pellets presented the crystallization of the structure of BG in renanite (JCPDS #29-1193) and sodium calcium silicate ($Na_3Ca_6(PO_4)_5$, JCPDS #11-236), CSP pellets did not show any phase on the XRD other than the β-TCP. This result supports the findings of Taveri et al. [2], in which 45S5 BG did not show any crystallization when consolidated by CIP. On SBF, CSP pellets showed greater value of pH on every measuring point when compared to TS pellets, indicating greater ionic release due to the non-crystalline BG structure. Both CSP and TS pellets showed morphological changes on the SEM after 7 and 14 days of immersion on SBF, which is an indicative of apatite formation.
Conclusions: CSP has shown to be suitable to consolidate β-TCP/BG composites, showing even higher densification than TS. Contrasting with TS, pellets consolidated by CSP did not present crystallization of the structure of the BG. Thus, CSP is a promising alternative for consolidating β-TCP/BG composites, but there are still many aspects to be explored, such as the influence of temperature and pressure in their densification, the use of different sintering media and β-TCP/BG ratios, and the combination of different calcium phosphates and BGs.

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CHARACTERIZATION OF ALGINATE HYDROGEL FOR OSTEOARTHRITIS TREATMENT

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Keywords: Cartilage; alginate; hydrogel; osteoarthritis.

Introduction and objective: Osteoarthritis (OA) is a degenerative joint disease that results in pain, inflammation, and progressive loss of joint function. Currently, available treatments aim to relieve symptoms but cannot interrupt or reverse the degenerative process. In this context, biomaterials have emerged as a promising therapeutic approach for OA. In particular, alginate (ALG)-based hydrogels have garnered great interest due to their biocompatible properties, and ability to encapsulate and release bioactive substances. This study aims to characterize an ALG-based hydrogel as a therapeutic intervention for OA, and evaluate the metabolic activity of fibroblasts cultured in indirect contact with the hydrogel.

Methodology: The hydrogel was prepared by dissolving ALG (3%) in distilled water at 65°C with magnetic stirring and adding $CaCO_3$ (3%) as a crosslinking agent [1]. GDL served as a solubilizer for $CaCO_3$ at ALG:GDL ratios of 4:1, 5:1, and 6:1 (G1, G2 and G3, respectively). Gelation time was determined by observing the flow of the ALG solution in microtubes every 30 seconds. Degradation assay involved incubating the hydrogel in PBS for 1 to 30 days. pH changes were evaluated at 0 to 90 minutes. Metabolic activity was assessed using the indirect contact assay with L929 fibroblasts, measuring alamarBlue® reduction.

Results and discussion: In the gelation test, the G1 group showed a shorter gelation time compared to G2 ($p=0.0104$) and G3 ($p<0.0001$). In the degradation test, the hydrogels initially exhibited a mass gain, followed by a mass loss relative to the initial weight. In this context, G3 showed a lower mass compared to G1 ($p=0.0033$) and G2 ($p=0.0050$) at the first evaluated moment, G2 had a higher mass than G1 ($p=0.0416$) at the second evaluated moment, and G1 had a higher mass compared to G3 ($p=0.0448$) at the fourth evaluated moment. It was observed that in G2, the pH stabilized at 15 minutes ($p=0.0270$), while in G1 and G3, it stabilized at 30 minutes ($p=0.0038$ and $p=0.0097$, respectively). In the metabolic activity test, G1 exhibited higher metabolic activity compared to control group (GC) ($p=0.0005$), an increase in metabolic activity in GC, G1, and G3 in the third experimental period compared to the first ($p=0.0115$, $p=0.0273$, and $p=0.0683$, respectively), as well as an increase in activity between the third and second moment for G3 ($p=0.0311$).

Conclusions: The results suggest that G1 appears to be the most favorable choice for further studies. It demonstrated a safe gelation time, good degradation properties, pH stability, and a higher metabolic activity compared to GC, suggesting enhanced cellular function.

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DEVELOPMENT AND CHARACTERIZATION OF ALIGNED NANOFIBERS USING A VARIANT OF ELECTROSPINNING CONFIGURATION

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Keywords: electrospinning; fibers alignment; anisotropic; tissue engineering

Introduction and objective: The electrospinning technique has been widely used in the production of nanofibers due to its easy implementation and its effectiveness in manufacturing micro and nanometric fibers. The static configuration of the electrospinning device allows obtaining randomly distributed fibers [1]. However, alignment methods to obtain fibers with controlled anisotropy have been proposed for application in several fields, including tissue engineering. One of them is the electrospinning gap in static configuration that allows electrospun fibers through two parallel electrodes [2]. In this work, random and aligned nanofibers were produced and their morphologies and anisotropic properties were compared.
Methodology: Two solutions of polyvinyl alcohol (PVA) (Serquim S.A) at 8% and 10% w/v heated at 60°C for 3h were prepared. Electrospinning parameters were set as follows: solution flow rate of 1 mL/h, voltage 10 kV, needle to collector distance of 10 cm. The fibers alignment were made using a parallel electrodes collector with a 1 cm gap between themselves and the random fibers were electrospun upon an aluminium plate collector. The morphology was observed by scanning electron microscopy (SEM) and the fibers alignment degree were analysed using the Fourier transform (FT) to estimate the degree of fibers anisotropy.
Results and discussion: Both, SEM images of aligned and random fibers were analysed using the ImageJ software. The diameters of randomly electrospun fibers were (440 ± 13) nm and (415 ± 73) nm for the 8% and 10% w/v solutions, respectively. The aligned fibers diameters were (324 ± 79) nm and (414 ± 73) nm for the 8 and 10 % solutions, respectively. FT result showed alignment degree of 0.774 and 0.829 for the parallel electrode collector fibers of 8% w/v and 10% w/v solutions, respectively. The diameters were between 5% and 22% smaller than random fibers, considering the same electrospinning conditions. On the other hand, the coherence degree of fibers obtained from the same solutions on the simple collector was 0.056 and 0.153, respectively. Taking into account that the maximum coherence or the maximum alignment degree from FT corresponds to the value 1, we corroborate the presence of anisotropy in the fibers obtained using the parallel electrode collector.
Conclusions: In conclusion, we can say that the parallel electrode collector was a simple variation in the electrospinning system that allows obtaining anisotropic fibers, also was observed that, the FT analysis is a reliable method to evaluate the alignment degree and preferential orientation of the fibers.

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EVALUATION OF BACTERIAL SENSITIVITY TO CHICHÁ GUM HYDROGEL ASSOCIATED WITH NEROLIDOL

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Keywords: Biomaterials; Essential Oil; Sterculia striata.

Introduction and objective: There has been a growing interest in polymeric materials with antimicrobial properties. Chichá gum, obtained from the Sterculia striata plant, is a notable example. This substance is a polysaccharide that contains hydroxyl groups throughout its structure, enabling the formation of hydrogels [1]. This characteristic allows for its use in drug delivery systems, and it can be combined with other materials, such as Nerolidol, to enhance its biological properties [2]. This study aimed to synthesize hydrogels using chichá gum and Nerolidol and evaluate their antibacterial activity.
Methodology: The chichá hydrogel was prepared by reacting isolated chichá gum with distilled water in a ratio of 1.5 g of chichá gum to 98.5 mL of distilled water. The solution was magnetically stirred for 30 minutes, adding 2 mL of Nerolidol and an additional 30 minutes of stirring. The hydrogels were characterized using Spectroscopy in the Infrared Region (FTIR). The antibacterial activity was evaluated using the direct contact method against *Staphylococcus aureus* and *Escherichia coli* strains.
Results and discussion: The FTIR analysis of the hydrogels indicated interactions between the gum and Nerolidol during the gel formation, with the presence of a specific compound in the regions of 3100-3550 cm−1. When evaluating the antibacterial activity, it was observed that the presence of Nerolidol in the hydrogels provided a more effective action against Staphylococcus aureus and Escherichia coli strains compared to hydrogels without Nerolidol and isolated Nerolidol. The chichá gum hydrogel with Nerolidol exhibited an antibacterial effect of 83.6% against the S. aureus strain and 37.4% against *E. coli*. Due to the lack of an additional permeability barrier in the cell wall, Gram-positive bacteria are more susceptible to inhibition of colony growth in direct contact tests, thus showing excellent antibacterial activity against *S. aureus*. It is because the cell wall of Gram-negative bacteria is rich in polysaccharides, which hinders the penetration of antimicrobial substances.
Conclusions: The materials were synthesized efficiently, and the characterization technique confirmed the presence of Nerolidol in the chichá hydrogels, as verified by Fourier Transform Infrared spectroscopy. The antimicrobial tests demonstrated that the chichá hydrogel associated with Nerolidol exhibited inhibitory activity against S. aureus and E. coli, making it a viable candidate as an antimicrobial agent and promising for biomedical applications.

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OBTAINING ANTIBACTERIAL MATERIAL BASED ON CHLORHEXIDINE AND CASSAVA GUM

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Keywords: Antibacterial action; Biomaterials; Hydrogel.

Introduction and objective: The search for natural materials that interact effectively with antibacterial pharmacological molecules has been carried out [1]. Among these molecules, chlorhexidine stands out. However, the main obstacle to the use of chlorhexidine is its rapid release [1]. Thus, the interaction of this drug with polymeric materials such as natural polysaccharides, for example, cassava gum, can provide stability and a more controlled release. Thus, this work aimed to obtain materials based on chlorhexidine digluconate and cassava gum. Structural characterizations and the antibacterial action of the materials obtained against Staphylococcus aureus and Escherichia coli bacteria were evaluated.

Methodology: Cassava gum hydrogel (CGH) was synthesized according to a previous study [2]. Chlorhexidine digluconate (CLX) was incorporated during the hydrogel synthesis, adding 10 mL of solution in 0.1 and 0.5% content, and named CGHC0.1 and CGHC0.5, respectively. The hydrogels were characterized by X-ray diffraction (XRD) and Fourier-transformed infrared (FTIR). For the antibacterial evaluation, the test used was the direct contact test [1] against the bacteria *Staphylococcus aureus* and *Escherichia coli.*

Results and discussion: XRD patterns for cassava gum (CG) show three peaks at around 2θ = 15, 17, 18, and 24 º that confer a semicrystalline characteristic to the CG. The synthesized hydrogels showed a crystallinity pattern different from the CG with a broad signal at around 2θ = 22.5°, which is associated with the amorphous nature of its polymeric matrix and the modification in the crystalline region of starch and the presence of polyacrylamide. For the samples with CLX, the presence of this molecule in different concentrations in CGHC0.1 and CGHC0.5 did not interfere with the material's crystallinity, maintaining an amorphous profile. Fourier- transformed infrared (FTIR) spectra show that the absorption bands present in the CG are characteristic of starch. In materials containing the drug, the emergence of new bands confirms the incorporation of CLX. Cassava gum hydrogel did not show antibacterial activity against both strains. However, chlorhexidine materials (CGHC0.1 and CGHC0.5) showed 60, 20, 100, and 45% activity against *S. aureus* and *E. coli*, respectively.

Conclusions: The FTIR and XRD characterization techniques confirmed hydrogel formation and chlorhexidine (CLX) incorporation. The results of the antibacterial activity of hydrogels with CLX showed better activity against *S. aureus* than against *E. coli* in used CLX concentrations, indicating the greater sensitivity of Gram-positive bacterins to the formed CLX-dense hydrogel.

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STUDY OF THE DEGRADATION OF 3D PRINTED SCAFFOLDS BASED ON THE PLDLA-TMC TERPOLYMER IN DIFFERENT RATIOS FOR BIOMEDICAL APPLICATIONS

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Keywords: bioreabsorbable; polymer; tissue engineering; 3D printing; degradation.

Introduction and objective: Synthetic bioreabsorbable polymers are widely used as orthopedic devices due to their capacity to undergo hydrolysis, generating non-toxic products that can be metabolized and eliminated from the body, and biocompatible. The advantage of the terpolymer poly(L-D,L-lactic acid-co-trimethylene carbonate), PLDLA-TMC is combination the favorable mechanical properties of poly(L-D,L-lactic acid) with enhanced flexibility and deformation provided by TMC, along with adjustable degradation time for biomedical applications. The objective of this study was to evaluate the degradation characteristics of PLDLA-TMC scaffolds in the ratios of 70:30 and 50:50, obtained through 3D printing, for tissue engineering applications.
Methodology: The PLDLA-TMC was synthesized in 2 TMC proportions: 30% and 50%, resulting in PLDLA proportions of 70% and 50%, respectively. The material was obtained in pellets format. The polymeric scaffolds were obtained using the 3D fiber deposition technique, employing the 3D-Bioplotter® system. The scaffolds were evaluated for *in vitro* degradation over time periods of 4, 8, and 12 weeks, using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and gel permeation chromatography (GPC).
Results and discussion: Based on the results obtained by SEM, the 3D printed PLDLA-TMC scaffolds showed that the designed pores experienced a decrease in their diameter due to water absorption by the scaffold fibers. A gradual surface erosion proportional to the immersion time was also observed [1]. The 50:50 ratio scaffolds exhibited rapid geometric deformation during the degradation period, resulting in the loss of porous interconnectivity after 8 weeks. GPC analysis demonstrated a reduction in chain size and number after 3D printing. The 50:50 ratio samples experienced a significant mass reduction, completely losing their 3D structure after 12 weeks. The material's glass transition temperature (Tg), as determined by DSC analysis, remained unchanged with processing, but after 12 weeks of degradation, the Tg of the 70:30 ratio was significantly reduced, while the 50:50 ratio experienced an even more pronounced decrease [2]. TGA results indicated that the scaffold processing conditions reduced the thermal stability of the materials. Both the 70:30 and 50:50 pellets showed a decrease in initial temperature and maximum decomposition temperature values after the scaffold 3D printing.
Conclusions: The TMC units added to PLDLA influenced the molecular weight and thermal properties, providing greater flexibility to the copolymer. Both scaffolds exhibited mass loss and thermal stability reduction during the hydrolytic degradation process, with the 50:50 ratio being suitable for soft tissue applications and the 70:30 ratio suitable for bone and cartilage applications.

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Mg ZX10 FOR PERIPHERAL NERVE REGENERATION: COMPARATIVE CORROSION STUDIES UNDER SIMULATED BIOLOGICAL CONDITIONS

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Keywords: Peripheral neuropathies; magnesium-based alloy; biodegradability; sterilization process.
Introduction and objective: Peripheral neuropathies are neurodegenerative diseases caused by dysfunction of one or more peripheral nerves. Magnesium (Mg)-based alloys emerge as potential biomaterials for the construction of nerve guide conduits. The primary constraint associated with this element is its high corrosion rate in the physiological environment. Moreover, the sterilization process and its impact are frequently overlooked in material research studies, despite its crucial nature. Sterilized and non-sterilized Mg ZX10 alloy were studied. Its degradation was evaluated in SBF (simulated physiological solution) and in DMEM (Dulbecco's modified Eagle's medium) high glucose with 10% fetal bovine serum (S) and its cytocompatibility was analyzed. **Methodology:** The microstructure of the Mg ZX10 alloy (Magnesium Innovation Centre, Germany) was analyzed. The hydrophilic character of unsterilized (control) and sterilized (oven at 180º 1h) alloy was determined by measuring the contact angle and their surface energy was calculated. Subsequently, both groups of samples were immersed in SBF (37ºC) and the sterilized samples were immersed in DMEM+S (37ºC and 5% CO_2) for 1 and 7 days. The alloy evolution was evaluated using: Raman spectroscopy, FTIR-ATR, electrochemical assays and scanning electron microscopy (SEM). Additionally, bone marrow mononuclear cells from rats were cultured varying concentrations biomaterial extracts, followed by MTT assay.
Results and discussion: The Mg ZX10 microstructure revealed a homogeneous grain distribution. The control and sterilized samples exhibited hydrophilic surfaces with surface energy values in agreement with previous reports [1]. Upon immersion in SBF, Raman spectroscopy detected the presence of magnesium oxides-hydroxides, phosphates and carbonates compounds in both groups of samples without significant differences. FTIR spectra of the sterilized alloys, after immersion in DMEM+S, showed the presence of amide and carbon- carbon bonds, magnesium-oxides, as well as phosphates and carbonates groups. These findings suggested the deposition of organic and inorganic compounds on the alloy surface. Electrochemical tests showed a significant reduction in the oxidation process of the sterilized alloy immersed in DMEM+S compared to that observed in SBF. SEM images showed that the alloy, previously immersed in SBF, exhibited degradation cracks. On the contrary, the sterilized alloy surface immersed in DMEM+S was more homogenous with deposit of structures similar to nanoflowers [2]. After 3-day culture, increasing extract concentration resulted in decreased cell viability. However, after 7 days, there were no significant differences in cell viability between the extract- exposed cells and the control group. Similarly, there were no notable differences in cell viability among different extract concentrations.
Conclusions: The sterilization process does not significantly change the surface characteristics of Mg ZX10. After alloy immersion in DMEM+S, deposits of organic-inorganic components with nanoflowers morphology were observed. Contrarily, in SBF the Mg alloy typical degradation was evidenced. This underscores the importance of interpreting the findings within the context of the specific analyzed system. Still, these studies could serve as a foundation for potential surface modifications in order to optimize Mg-alloy properties.

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DEVELOPING AND CHARACTERIZING A BIOABSORBABLE MEMBRANE TO COMBAT INFECTION IN ORTHOPEDIC IMPLANTS

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Keywords: Drug Delivery System, bioabsorbable Polymer, biomaterials, implant infection.

Introduction and objective: Infections are a significant complication in implant surgeries, with about 20% of orthopedic implants failing due to bacterial infection. Systemic treatment of these infections often requires high drug doses to achieve sufficient local therapeutic levels. Drug delivery systems offer a promising approach to enhance the local concentration of antibiotics, effectively combating local infections. The efficient antibiotic, rifampicin, is known for its ability to combat biofilms. This study focused on developing and characterizing a bioabsorbable polymeric matrix Drug Delivery System, utilizing PLDLA. The aim was to couple it with orthopedic implants, thereby minimizing the risk of local infection.

Methodology: The proposed anti-infection system (AIS) is based on bioabsorbable polymer membranes of PLDLA (PURUSORB® PLDL 7038, poly L-lactide/D-L lactide, Carbion PURAC) loaded with Rifampicin. The solvent evaporation method was employed to obtain these membranes. Solutions of PLDLA and chloroform (Synth) at a concentration of 1% w/v were used for the fabrication of membranes, both with and without the antibiotic. The drug concentration used was 1% and 0.5% w/w relative to the polymer's mass. Subsequently, the membranes were characterized using the following techniques: Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and Fourier Transform Infrared Spectroscopy (FTIR).

Results and discussion: The produced membranes had a thickness ranging from 10 to 50 micra. FTIR results confirmed the presence of characteristic rifampicin groups in the PLDLA membrane, particularly at 1654 cm^{-1} (C=N) and a discrete signal at 3418 cm^{-1} corresponding to the drug's OH group. The DSC analysis of rifampicin exhibited an endothermic event at around 192°C, associated with its melting, followed by a thermal event related to recrystallization, at approximately 209°C. Morphological analysis of the membranes through SEM revealed a dense appearance for both membranes containing rifampicin and those without it. TGA analysis of the membrane containing rifampicin indicated an onset of mass loss at a temperature close to 250°C. The use of rifampicin is intriguing as it belongs to a group of antibacterial agents effective against most Gram-positive and some Gram-negative microorganisms, including Escherichia coli, which may frequently occur in the immediate post-operative period.

Conclusions: The work allowed obtaining PLDLA membranes containing or not the drug rifampicin. The characterization of the produced membranes was performed, which represents a fundamental step to enable their subsequent use as coatings for prostheses. The goal is to inhibit/combat bacterial infection processes that may occur in such devices.

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DEVELOPMENT AND CHARACTERIZATION OF HYDROGEL FOR USE IN KNEE JOINT DISORDERS

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Keywords: biomaterial; hydrogel and knee joint disorders;

Introduction and objective: Cartilaginous tissue has the ability to remodel throughout life, however, this capacity is exceptionally reduced in adults, mainly because it is an avascular and hypocellular tissue. Due to the overload naturally imposed on the knee joint, it is one of the most affected by different injuries, thus arousing the interest of researchers in innovative treatments such as hydrogels that have similar characteristics to the extracellular matrix present in cartilage. So, within this context, the objective of this study was to develop a hydrogel and alginate, characterize their physicochemical properties, and evaluate their compatibility for use in knee joint disorders.
Methodology: The hydrogel was manufactured by dissolving alginate (1.5%) and calcium carbonate (1.5%) in distilled water under agitation at 65ºC. After mixing these compounds, glucolactone (GDL) was added in different proportions: 4:1; 5:1, and 6:1 (ALG:GDL), resulting in 3 experimental groups, G1, G2, and G3, respectively. The characteristics of gelling time, pH change, degradation rate, and cell viability were evaluated as described below:

- Gelling Time: record the time the hydrogel did not flow in the microtube [1].
- pH change: 0; 6; 15; 30; 60; and 90 minutes [2].
- Degradation rate: 1; 3; 6; 12; and 30 days
- Cell viability: metabolic activity of L929 cells at 1, 3, and 6 days

Results and discussion: The analysis of the results proved that the G3 presented a longer gelation time when compared to the G1 and G2. The pH stabilization occurred after 15 minutes in G1 and 60 minutes in G2 and G3. All groups of hydrogels showed an increase in mass in the first four evaluated moments, followed by a decrease in the last evaluated moment in relation to the initial weight. Although the metabolic activity did not differ between the groups at different times, there was an increase both in G2 when comparing the second with the third moment, and in all groups when comparing the first with the third moment evaluated
Conclusions: The results found in this study allow us to state that, among the hydrogels studied, the one that presented the best characteristics to be administered in the knee joint was the one with the smallest volume of GDL. This biomaterial presents rapid pH stabilization, a longer gelling time, and adequate degradability, in addition to being biocompatible in *in vitro* studies.

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MECHANICAL CHARACTERIZATION OF ALG/RGO/TA HYDROGELS

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Keywords: Alginate hydrogel, tannin, reduced graphene oxide, rheology

Introduction and objective: Hydrogels are 3D polymeric structures with the ability to retain water in addition to having great flexibility, biodegradability, biocompatibility, and mimicking properties of different tissues in the body, making them ideal candidates for biomedical applications, but their use is limited by deficiencies in mechanical properties. In this study, reduced graphene oxide (rGO) and tannins (TA) were introduced into the polymeric matrix of alginate (Alg) to evaluate the influence of the composition and concentration of rGO and TA on the mechanical properties under rheological characterization.
Methodology: Hydrogel synthesis was carried out by adding glycerine (4 mL) and $FeCl_3$-6 H_2O (42 mg) in Milli Q water (42 mL); then rGO and TA were added in different mass proportions (0%, 4.5% and 9%). Alg was the control. The samples were deposited in molds and dried. The sample`s viscoelastic properties were assessed with a TA Instrument rheometer with a Peltier plate for temperature control. Oscillatory strain sweep assays were carried out to determine the linear viscoelastic region and the yield strain. The storage moduli G' and loss moduli G" were obtained. A parallel plate geometry was used.
Results and discussion: The amplitude sweeps allowed identification of the linear viscoelastic zone (LVR), enabling a determination of the critical deformation point for each sample. The Alg hydrogel presented a critical deformation point at 32.3% deformation. The addition of rGO had a great effect on this property, increasing this percentage to 102.4 % (Alg/$rGO_{4.5}$) and 90.8 % (Alg/rGO_9). The inclusion of TA also showed an increase in the critical deformation point, it being 88.2 % (Alg/$TA_{4.5}$) and 82.2 % (Alg/TA_9). When analyzing Alg/rGO/TA hydrogels, it was observed that, for a fixed TA concentration, as the rGO concentration was increased, the properties tended to increase, or no significant changes were observed. Frequency sweeps were performed within LVR at 1% strain. All the hydrogels showed, in the entire frequency range, G' values greater than G'', indicating the formation of a viscoelastic gel. On the other hand, a slight dependence of G' and G'' with the angular frequency was observed, which is a representative behavior of hydrogels, dependence being greater in the Alg/rGO, Alg/TA and Alg/rGO/TA hydrogels. with respect to the Alg hydrogel.
Conclusions: The rheological characterization confirms the formation of hydrogels, and, by introducing rGO and TA, it was possible to increase the strength of Alg hydrogels.

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PRE-TREATMENT OF POLYMERIC SAMPLES FOR WETTABILITY TESTS

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Keywords: biomaterials; ABS, PCL, PLA, laser, wettability.

Inroduction and objective: This work presents the pre-treatment of polymeric samples [ABS (acrylonitrile butadiene styrene), PCL (polycaprolactone), PLA (polylactic acid)] to carry out wettability tests. In the area of materials for use in biomedical engineering, the hydrophobic behavior is one of the most relevant characteristics[1].To carry out the contact angle tests, the samples of polymers made by 3D printing were subjected to a pre-treatment to smooth the contact surface.
Methodology: For the black colored PLA samples, an Infrared Laser Diode equipment was used, at a wavelength of λ = 940 nm, with a scanning speed of 500 mm/s, at a power of 8 W, with 5 sequential repetitions. The same conditions were used for the brown colored PC samples. All the samples were subjected to a constant pressure of 2 bar, exerted between the base and the upper glass of the support in the equipment chamber. As the laser action occurs at the contact interface between the sample and the glas, the locally heated sample conforms to the smooth surface.
Results and discussion: The orange colored PCL samples and the white colored ABS samples were transparent to the wavelength of the IR laser used; then these samples were subjected to a heat treatment to achieve the objective of surface smoothing. The samples were pressed, at a constant 2 bar, between a thermoelectric base and the glass of the laser chamber; the temperature of this base was adjusted to the ABS Tg (approximately 105º C) and cooled after 10 to 15 min to remove the samples. As the Tg of PCL is of the order of -60º C and its melting temperature 60º C, a temperature close to the melting point was used for these samples and the same pressure of 2 bar was applied for a few seconds. With the thermal treatment described, surfaces as smooth as those provided by laser treatment on the first samples can be achieved.
Conclusions: With the "rectified" surfaces, we will be able to carry out wettability tests, with the aim of comparing the behavior of the polymers before and after the superficial laser treatment planned.

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MICROSTRUCTURAL ANALYSIS OF POLYCAPROLACTONE AND ATORVASTATIN NANOCOMPOSITES

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Keywords: hot melt extrusion (HME); drug-delivery; 3D-printing

Introduction and objective: Hot melt extrusion (HME) is a process of mixing and heating a carrier in its molten state and a given drug, giving rise to a solid dispersion in which the active pharmaceutical ingredient can be dissolved or dispersed in the medium. Atorvastatin (ATV) is a statin with pleiotropic effects in bone metabolism and vasodilatation. Moreover, it has action immunosuppressive, antioxidant, and anti - inflammatory. It should be also highlighted that ATV is used in cardiovascular diseases. This work aims to evaluate the microstructure of nanocomposites of Polycaprolactone (PCL)/ATV produced by HME employing X-Ray Diffraction (XRD) and Small Angle X-Ray Scattering (SAXS).
Methodology: The PCL-ATV nanocomposite (PCL_ATVn) and PCL filaments (PCLf) were produced by HME at 60◦C in a mono-screw extruder (Filamaq-3D). The samples were analyzed on a D8 Discover X-ray diffractometer from Bruker. The scans were recorded in the 2Θ range of 5 to 60 °, with a step size of 0.02 ◦ and 2s per step. The software Topas (Bruker) was used to fit the peaks of the samples PCLf and PCL_ATVn, and it was possible to obtain the mean crystallite size. SAXS analyses were performed on a Bruker Nanostar. The SAXS data were recorded for 3 h under vacuum.
Results and discussion: XRD results revealed that ATV caused a modification in the crystalline structure of PCL. PCL_ATVn showed more defined and less intense peaks than the PCLf sample. The two most intense peaks of PCL_ATVn (2Θ =21.7 ◦ and 22.4 ◦) are related to PCL. The extra peak at 24.1 ◦ is, then, probably related to ATV. No sharp peaks of ATV were observed in PCL_ATVn, so it can be suggested that there was a loss of ATV crystallinity when it was added to PCL. The mean crystallite sizes of PCLf and PCL_ATVn were 15 and 17 nm, respectively. According to SAXS results, PCL_ATVn shows the major peaks of PCLf and the one of ATV, both with less intensity than the pure samples peaks. The interplanar distance (d) for polymers can be related to the interlamellar distance. The peak of PCLf at 0.11 Å -1 (57 Å) is shifted to 0.13 Å -1 (48 Å) in the PCL_ATVn. This shift is due to the intercalation of ATV in the PCL lamellae. SAXS results suggest that ATV caused a difference in the crystallinity, probably due to the presence of small ATV domains intermixed with the polymer. SAXS results agree with the XRD observations.
Conclusions: In the present work, PCL_ATV nanocomposites were evaluated by XRD and SAXS. The results indicate that the HME process promotes alteration in the crystallinity of both the polymer and the drug. The SAXS analysis shows the presence of ATV in the sample. It suggests that there was an intercalation of ATV between the polymer lamellae, which corroborates the formation of a nanocomposite.

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OLANZAPINE CRYSTALLIZATION IN PLA-BASED MATRICES FOR SUBCUTANEOUS IMPLANTS: A MICROSTRUCTURAL ANALYSIS USING X-RAY DIFFRACTION

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Keywords: olanzapine; biomaterials; PLA; drug-delivery

Introduction and objective: Olanzapine (OLZ) is an atypical antipsychotic prescribed to manage certain psychiatric disorders, including schizophrenia and bipolar disorder. Incorporating OLZ in polymeric devices, such as rods produced by hot melting extrusion, has been studied to produce subcutaneous biodegradable implants, a potential alternative to improve the bioavailability of the drug in patients with schizophrenia. PLA (polylactic acid) is a commonly used biomaterial for drug delivery applications. In the present work, matrices of PLA contained OLZ were produced by solvent casting and compression molding. This work aims to evaluate the microstructure of the obtained materials employing X-Ray Diffraction (XRD). [1]

Methodology:

Matrices of PLA/OLZ (10% wt of OLA) were produced by compression molding (PLA/OLZ-CP) at 164oC for 5 minutes) and solvent casting (PLA/OLZ-SC, using chloroform as solvent at 56 C). All samples were dried at 25oC. To evaluate the crystallinity pattern, the analysis of PLA/OLZ-CP, PLA/OLZ-SC, and pure materials (OLZ and PLA) were performed on an X-ray diffractometer (Rigaku, model Mini Flex II) operated with the Cukα source (λ = 1.5418 Å). The scans were recorded over the range of 2θ° = 6–60°, with a scan speed of 2°/min.

Results and discussion:

In the diffractogram of pure OLZ, peaks at 2(θ°) equal to 9.12°, 10.62°, 13.02°, 14.12°, 14.82°, 17.14°, 18.56° and 19.9° were observed. The main peaks of PLA are 16.9° and 19.18°. A reduction of the amorphous halo, related to PLA, was also observed. Comparing the PLA/OLZ-CP and PLA/OLZ-SC, it is possible to state that the methodology also affects OLZ and PLA patterns. In PLA/OLZ-CP diffractogram is possible to observe the main OLZ's characteristic peaks and the peaks related to PLA. In PLA/OLZ-SC the OLZ's peak more visible was 10.62°. The PLA/OLZ-CP formation was based on PLA melting. The PLA/OLZ-SC production involved the OLZ and PLA solubilization. The OLZ solubilization occurred prior to PLA. Due to it, the PLA's polymer chains were swollen in the presence of solubilized OLZ. The evaporation process allowed, possibly, the intercalation of PLA and OLZ's molecules. As a result, the diffractogram did not show all the OLZ's peaks, indicating that the methodology interferes with the OLZ's crystallization process. A reduction of the amorphous hallo, related to PLA, was also observed in PLA/OLZ-SC and PLA/OLZ-CP. The PLA, PLA/OLZ-CP and PLA/OLZ-SC's crystallinity were 6,5%, 22,8% e 23%,respectively.

Conclusion: In the present work two methodologies were evaluated to produce matrices contained OLZ. One based in the polymer melting process and other based on the polymer and drug solubilization. In both techniques employed, the resulting matrices presents cristallinity major than the pure PLA. Futhermore, the use of solvent, PLA/OLZ-SC, shows considerable changes on the patterns of OLZ's cristallinity than the heated method. Those changes can also be observed in Almeida's (2021) work. However, PLA's cristallinity was improved.

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INNOVATIVE STRATEGIES WITH IRON OXIDE NANOPARTICLES TO ERADICATE BACTERIAL BIOFILMS ON IMPLANTABLE BIOMATERIALS

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Keywords: nanoparticles;magnetite; bacterial biofilms

Introduction and objective: Multi-drug resistant bacteria have turned bacterial infections into a great threat to public health and the economy, which is why it is necessary to develop new methods for their eradication. The objective of this work is to develop superparamagnetic iron oxide nanoparticles (SPIONs), with an environmentally friendly synthesis method [1], to eradicate bacterial biofilms on implantable materials. The eradication of biofilms can be carried out by magnetic force, complemented by the antimicrobial effect of phytocompound capping or in association with conventional antibiotics. The SPIONs must have low cytotoxicity, antimicrobial action, adequate magnetic response, feasibility of resuspension, and stability.
Methodology: Nanoparticles (NP) were synthesised by electrodeposition from Fe(II) and Fe(III) salts and coated using tannic acid (TA) at different pHs (alkaline, neutral and acid) in aqueous medium. The characterization of the SPIONs obtained was carried out using vibrating sample magnetometer (VSM), Mossbauer effect spectroscopy, X-ray diffraction (XRD), dynamic light scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR) and thermogravimetric analysis (TGA). SPION cytotoxicity analysis was performed using the dose-response curve by methyl tetrazolium (MTT) reduction assay with MC3T3E.1 pre-osteoblastic cells. Preliminary bacteriological assays with *Staphylococcus aureus* were also performed.
Results and discussion: The characterization of the SPIONs by Mossbauer and XRD confirms the obtaining of magnetite/maghemite NPs, with presence of metallic Fe. The VSM measurements show a superparamagnetic behaviour and a saturation magnetization of ~120 emu/g. This last is associated with the metallic Fe.
The TA coating was confirmed by FTIR (SPIONs@TA). The alkaline TA-coating allowed better dispersibility in water and stability of the suspensions compared to NPs with neutral and acidic coatings. DLS measurements of SPIONs@TA at alkaline pH distinguish two populations, ~30 and 250 nm. SPIONs@TA at alkaline pH presented low levels of cytotoxicity (viability >80%) for suspensions up to 100 µg NPs/mL (6.9 ug Fe/ml).
The TGA analysis of the NPs without capping is compatible with the magnetite NPs, showing an increase in mass from 400 °C that is explained by the oxidation of metallic Fe, possibly located in the core of the SPIONs. The TGA results for SPIONs@TA at alkaline pH confirm a higher anchoring efficiency than in the case of SPIONs@TA at other pHs.
The antimicrobial effect of SPIONs@TA was assayed against *S. aureus* and preliminary results suggested that other strategies, such as magnetic force or combination with antibiotics, should be applied.
Conclusions: SPIONs were obtained through eco-compatible technique as electrodeposit, with capping of the natural compound TA that gave them stability and low cytotoxicity. The presence of metallic Fe is possibly due to the formation of nuclei of this material covered by magnetite. TA coating at alkaline pH provides adequate physicochemical properties to SPIONs, which allows further microbiological and cytotoxicity studies.

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MECHANICAL CHARACTERIZATION, HYDROLYTIC DEGRADATION, AND CELL VIABILITY OF A NEW POLYURETHANE BASED ON PCL AND TRIMETHYLENE CARBONATE

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Keywords: Polyurethane; PCL-TMC; Characterization; Cell Viability

Introduction and objective: From the perspective of tissue engineering, the demand for devices with specific mechanical properties, as well as biodegradability and cytocompatibility features, has led the scientific community to continuously explore new biomaterials. Among the polymers extensively investigated for tissue engineering approaches, polyurethane holds a prominent position. For this reason, the present study aimed to characterize a new polyurethane with improved mechanical properties based on Polycaprolactone-diol (PCL-diol) and Trimethylene Carbonate (TMC) by tensile testing, evaluation of hydrolytic degradation, and cell viability assessment.

Methodology: To evaluate the tensile performance, the polyurethane was subjected to a mechanical tensile test, following the ASTM D882-02. The in vitro degradation analysis of the polyurethane was conducted over a 48-week period by measuring the mass loss of the polymer when it was immersed in PBS at 37°C. Cell viability was assessed by measuring mitochondrial activity through the MTT metabolic oxidation method. Additionally, Live/Dead® viability staining was used to analyze the ratio of living and dead mesenchymal stem cells (MSC) via laser scanning confocal microscopy (LSCM) after 1, 3, and 7 days of cell cultures.

Results and discussion: The Poly(PCL-TMC)urethane exhibited a deformation of 983.03 ± 289.20%, an elastic modulus of 2.62 ± 0.17 MPa, indicating the elastomeric nature of the material. Also, it irreversibly deforms at low stresses with a maximum yield stress of 0.61 ± 0.09 MPa, which is a common characteristic of copolymers containing between 25 and 75% TMC. No mass loss was observed during the 48-week in vitro degradation when immersed in PBS at 37°C, which is a well-known property of most polyurethanes due to their high resistance to hydrolysis. However, there are some exceptions, such as polyester-based polyurethanes, which are susceptible to hydrolytic degradation. Nevertheless, despite PCL containing ester groups in its composition, the hydrophobic nature of this polymer contributes to the greater hydrolytic stability of the material [1]. Calcein-AM and EthD-1 signals obtained by the Live/Dead® assay demonstrated that the MSCs were able to grow and adhere to the polyurethane substrate, which was consistent with the MTT results [2].

Conclusions: The tensile test showed that the polymer has a high elongation capacity and a low value of Young's modulus, demonstrating its potential for use in applications that require these characteristics. The hydrolytic degradation assay evidenced the polymer stability, and the cytocompatibility tests revealed high cell density after 7 days, indicating that the novel polymer obtained is a promising cellular carrier. Thus, the results showed that Poly(PCL-TMC)urethane has potential for elastomeric applications, including meniscal prostheses or cardiac devices.

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DECELLULARIZED EXTRACELLULAR MATRIX MEMBRANES DERIVED FROM BOVINE CORNEA: CHARACTERIZATION AND APPLICATIONS

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Keywords: Membrane; biomaterials; decellularized extracellular matrix; cornea

Introduction and objective: Tissue engineering in regenerative medicine and scientific research requires structures to rebuild tissues effectively. Key components in biological tissues, including the extracellular matrix, cells, and bioactive molecules, play crucial roles. The extracellular matrix provides support, cell adhesion, proliferation, and function, emphasizing the significance of biomaterials in creating a natural-like microenvironment for cell culture. Scaffolds can be made from diverse biomaterials, both biological and synthetic. Natural biomaterials, such as decellularized extracellular matrix, offer significant promise since they preserve all native tissue components, providing numerous advantages. The aim of the study is to develop a decellularized extracellular matrix hydrogel membrane derived from bovine cornea for application in ocular cell culture.

Methodology: In this study, bovine corneas were subjected to decellularization using SDS 1% and solubilization aided by chemical digestion (pepsin) and mechanical methods (tissue grinder) to obtain a hydrogel. The hydrogel was then placed in 6-well inserts with a PVC film membrane and dried in an oven at 37ºC for 24-48 hours. After drying, the PVC film was removed, leaving only the extracellular matrix membrane. The structure of the membranes was characterized using Scanning Electron Microscopy (SEM). To prepare the membranes for cell culture, they were sterilized using UVA light and hydrated with a DMEM culture medium. Primary human corneal limbal cells and human keratinocyte lineage cells (HaCat) were cultured in advance. Both cell types were then seeded onto the membranes at a density of $5x10^5$ cells/well. The cell viability was assessed using the MTT method, and the cells were histologically evaluated using the Live/Dead immunofluorescence method. Additionally, for HaCat cells, Trans-Epithelial Electrical Resistance (TEER) was measured.

Results and discussion: The results of the study indicated the successful decellularization and solubilization of the matrix, effectively preserving the essential components of the extracellular matrix, including collagen and glycosaminoglycans. The process of obtaining the membrane was also deemed successful, as evidenced by the presence of observable fibrillar structures in scanning microscopy, both on the surface and within the fracture of the membrane. While cellular tests are still ongoing, promising preliminary results have demonstrated the membrane's cytocompatibility with both primary human cells and human lineage cells.

Conclusions: Indeed, the positive results of this study indicate that the developed membrane is very promising for primary and lineage cell culture applications. This finding paves the way for significant advances in the fields of tissue engineering, regenerative medicine, and scientific research focusing on ocular systems. By providing a suitable and cytocompatible microenvironment for cells, this membrane offers new avenues for reconstructing and regenerating ocular tissues such as the cornea, promoting progress in medical treatments and scientific investigations.

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PRODUCTION OF A DOUBLE-NETWORK HYDROGEL USING SODIUM ALGINATE AND NANO-STRUCTURED CELLULOSE TO 3D CELL CULTURES

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Keywords: Hydrogel, 3D cell cultures, Cellulose, Double-Network.

Introduction and objective

2D cell cultures have limitations regarding on tissue representativity. 3D cell cultures can use hydrogels of alginate with cellulose with adequate viscoelasticity properties for cell growth, being from plant sources, abundant and low cost. This work consisted of producing a biocompatible gel from plant sources for three-dimensional cultures, promoting polymeric matrices for cells, helping in cell interactions and nutrient transport, providing mechanical support, self-assembly capacity, biodegradation, ability to reticulation, stability control and mechanical resistance.

Methodology

Transformation of microcrystalline cellulose into nanofibers was achieved freezing aqueous suspensions on presence of 4M NaOH to proper dissociation of fibers. To obtain suitable dispersion, sodium citrate was added to prevent aggregation. Suspensions were analyzed by Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Zeta Potential. For cell viability analysis, murine fibroblastic cell lines (NIH/3T3) were plated (2.5 x 10^5 cells per well) 24-well plate embedded in gel (100µL).

Results and discussion

The analysis of cellulose suspensions through SEM, showed a significant change in the size and shape of the structures after hydrolysis, indicating the obtention of structures on a nanometric scale. For the analysis of cellulose aggregation, the zeta potential values indicated that after the addition of sodium citrate, greater dispersion was obtained between the cellulose structures, enabling resistance to the structure in a uniform way. FTIR analysis showed changes in the covalent bonds of the products. Cell viability assay showed structures containing fibroblast cells, alginate and cellulose with 1 cycle of freezing with citrate showed an intact gel structure, with cell aggregates indicating possible cell growth, while the one with only alginate showed dead cells and showed that the hydrogel did not induce cellular toxicity. These results suggest that the hydrolysis of microcrystalline cellulose can lead to obtaining cellulose nanofibers with potential for applications in tissue engineering.

Conclusions

Hydrogels, they have potential for applications in tissue engineering, since they have mechanical resistance and cell viability. In addition, hydrogels from exclusively vegetable sources, since these are in large quantity, low cost and environmental impact, given that the alginate comes from brown algae found in several coastal regions and the cellulose can be extracted from renewable sources or various vegetable waste from agroindustry.

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ANALYSIS AND CHARACTERISTICS OF ADVANCED CERAMICS AS A BIOMATERIAL FOR ORAL AND MAXILLOFACIAL USE

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Keywords: bioceramics characterization; advanced ceramics; maxillofacial; implant material.

Introduction and objective: Bioceramics are known for their raw material being of high purity and the processing of their products requires high control. Like aluminium oxide and zirconium oxide, they are examples of application in the medical-dental area and implant dentistry, due to their properties and good response to the physiological system. The objective was to analyse the characteristics of bioceramics (zirconia and ZTA), aiming at their use in oral and maxillofacial traumatology and the processing conditions of the products obtained.

Methodology: The analysis method starts with the evaluation of the raw materials in its elemental composition by X-Ray Fluorescence. Sintering temperature and pressing load in the hydraulic press was evaluated to evaluate the influence in mechanical properties. After sintering, tests were carried out, such as density and apparent porosity by Archimedes method, Vickers hardness and 4-point bending, in addition to the cytotoxicity of these bioceramics.

Results and discussion: Samples of zirconium oxide (zirconia) and zirconia toughened alumina (ZTA) had their elemental characterization by the x-ray fluorescence technique, where it is possible to confirm the chemical composition of the materials, with no other contaminant. One of the objectives of this work was to elucidate how the processing conditions would influence the mechanical properties. The experimental density for the zirconia samples was very close to the expected with apparent porosity close to zero. The sintering temperatures 1500°C, 1550°C and 1600°C also did not directly influence properties such as density and 4-point bending. The bending stress with value average 257 MPa for zirconia sintered at 1550°C and 184 MPa for ZTA at the same temperature. The average Vickers hardness of the samples presents a value close to that required in the ISO 6474-2 standard, greater than or equal to 15.5GPa. The cytotoxicity formulation was evaluated with primary human fibroblasts. Sulforhodamine B (SRB) assay was performed. The samples for this test were obtained through ISO 10993-12 and it was verified that there was no cytotoxic effect of the bioceramic on the fibroblasts, contributing to the use of the material as a medical device.

Conclusions: It is concluded that the ceramic samples of zirconia and ZTA obtained by the pressing process are able to reproduce characteristics of medical devices for dental use in accordance with internationally established norms and standards. Using experimental data to improve and adapt processing conditions to obtain products for medical purposes was an objective achieved with this study.

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BIOPLASTIC BASED ON CUCUMBER MESOCARP PUREE (*CUCUMIS SATIVUS*)

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Keywords: bioplastics, polymeric films, casting process

Introduction and objective. The packaging industry sends its largest share to the food sector with 51% and to the beverage sector 18%, cosmetics 20%, pharmaceuticals 6% and personal hygiene 5%. Within this sector, plastics are widely used due to some characteristics such as durability, transparency and good barrier properties, but they are highly harmful to the environment and are not sustainable. For this reason, there is a growing interest in the development of edible films and coatings based on biopolymers that can play a parallel role in protecting against deterioration and preserving flavor and aroma. For these materials to be part of the food to be consumed, the materials used in their formulations must be non-toxic and safe for human consumption [1]. The objective of this work was to obtain films from cucumber mesocarp (Cucumis sativus) using the casting process. This proposal is relevant, as the cucumber mesocarp is discarded during processing and is a renewable and biodegradable source.

Methodology. Cucumbers were purchased at city markets and washed to remove dust and debris from the skins. It was sliced into four parts longitudinally, the seeds and placenta were removed and the mesocarp was separated from the endocarp. The respective mesocarp was cut into cubes and hydrothermal treatment was applied. After cooling, the mesocarp were pre-crushed in a blender and processed in an Ultra Turrax highenergy dispersing element for 5 minutes at 7000 rpm. The processed puree is separated into two samples and named as C-NW and C-W. The C-NW is taken directly to dry in an electric hood for approximately 7 hours using the casting process. All compounds soluble are removed from the C-W puree and dried using the same process as for the C-NW [2]. The films are named with the corresponding name of puree.

Results and discussion. It is possible to obtain two different products derived from cucumber: C-NW and C-W films. The first is flexible and slightly yellowish in color and the second semi-transparent with less flexibility. Thermal stability was evaluated by Thermogravimetry (TG/DTG), indicating water loss (22.6-96.8°C) and thermal decomposition (96.8-150.5°C,150.5-232.8°C and 230.5-357.1°C) for C-NW and water loss (21.0-143.7°C) and thermal decomposition (143.7°C-253.3°C and 253.2°C-380.0°C) for CW. Fourier Transform Infrared Spectroscopy (FTIR) showed less intense bands for CW and absence of bands 863, 825 and 772 cm^{-1} for C-NW. Scanning Electron Microscopy (SEM) showed CW films with a dense surface with reliefs and C-NTW showed a surface with randomly intertwined fibers.

Conclusions. C-NW and C-W films have different properties because C-NW contains all soluble compounds, while C-W films do not. Based on these properties, it is possible to direct the C-NW films to the food area and the C-W films to the medical and pharmaceutical area.

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CORROSION ANALYSES OF ANODIZED ALUMINUM FOR BIOMEDICAL PURPOSES

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Keywords: biomaterials; nanoporous anodization; Al2O3; corrosion

Introduction and objective: Anodic aluminum oxide has attracted a lot of interest due to the regular arrangement of nanopores, ease of control of the nanopores diameter, large specific surface area, low cost, good thermal stability, absence of toxicity and biocompatibility. The geometric arrangement of nanopores makes it possible to use alumina as a mold for the synthesis of various nanostructures, such as nanopores, nanotubes, nanorods and nanowires that have many advantages in advanced application areas due to their unique chemical, physical, mechanical, and optical properties [1,2]. The objective of this work was to study the corrosion susceptibility of anodized aluminum samples for biomedical applications.
Methodology: In the present work, the localized corrosion resistance of AA6061aluminum alloy anodized in oxalic acid solution (C2H2O4) and sulfuric acid (H2SO4) was evaluated by electrochemical techniques. Prior to the anodization stage, the samples were electrolytically polished in a solution of perchloric acid and ethanol.
Results and discussion: All samples showed a protective behavior on their surfaces, higher corrosion potentials in relation to the standard reference sample and a shift towards lower values of corrosion current densities in relation to the sample without passivation treatment. These results indicate that the anodizing treatments of AA6061 aluminum surfaces in oxalic or sulfuric acid are effective in producing surfaces resistant to localized corrosion and can therefore be used to coat this type of surface, ensuring an increase in the useful life of the devices.
Conclusions: The results indicated superior corrosion resistance in the anodized samples in both conditions. Therefore, it is necessary to constantly advance research on the use of nanoporous anodic alumina coatings on biomaterials surfaces.

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SURFACE INVESTIGATION OF A LASER ETCHED METALLIC BIOMATERIAL

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Keywords: biomaterials; laser; 316L ss; electrochemistry

Introduction and objective: Surface treatments are used to improve characteristics, such as: markings, texturing and polishing. The texturizations are produced to provide roughness and, consequently, adherence in specific locations of implantable medical devices of permanent character, that is, implants of prolonged use. Sometimes this process can generate stress concentrators and regions with probability for the occurrence of failures that can lead to fracture; in addition to damaging the passive layer, favouring the initiation of various forms of corrosion [1]. This work aims to evaluate the effect of the laser beam texturing technique in metallic implants on the corrosion resistance of ASTM 316L stainless steel.
Methodology: Samples were prepared from the stainless steel textured by fiber optic laser doped with ytterbium (Yb) by changing the values of the frequency of the laser pulse cadence and keeping the other parameters constant. As a comparison, samples of the biomaterial without any type of laser treatment were also evaluated. The electrochemical tests performed consisted of open circuit corrosion potential (OCP) monitoring and cyclic potentiodynamic polarization measurements, determined after hours of immersion at 37°C body temperature. The scanning vibrating electrode electrochemical technique (SVET) was used as a tool to determine the corrosion current density in 0.1M NaCl solution.
Results and discussion: The results obtained revealed the highest anodic current densities in the regions engraved by the laser beam and cathodic current densities in the regions farthest from the engravings, which indicates that laser engraving, in addition to increasing the roughness of the surfaces, makes them essentially anodic, changes the passive layer, affects the distribution of corrosion current densities and decreases the resistance to localized corrosion of this biomaterial.
Conclusions: The change in the laser pulse frequency values is directly related to the behaviour observed on the analysed surfaces, indicating that the laser texturing treatment affects the passive layer of the material decreasing the resistance to localized corrosion.

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MAGNETIC PROPERTIES EVALUATION OF 316L STAINLESS STEEL PRODUCED BY ADDITIVE MANUFACTURING FOR BIOMEDICAL USE

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Keywords: biomaterials; laser; 316L ss; magnetics

Introduction and objective: The modern additive manufacturing (AM) techniques represent the current state of the art of industry 4.0. Advanced selective laser melting techniques allow the production of parts with the most varied sizes, shapes and complex geometries, which would be difficult to obtain previously with casting, joining, machining, among others. In addition to saving material, they are automated, do not generate wear to the tooling and little waste. The durability of surgical instruments, implants, and prostheses with this type of manufacturing can be considered greater than that using conventional methods with cutting tools [1]. Austenitic stainless steels have been widely used for the manufacture of implants due to their good mechanical and electrochemical properties and their relative low cost. The present work evaluated the variation of some laser beam conditions, regarding the magnetic susceptibility in AISI 316L stainless steel samples produced by additive manufacturing (AM).
Methodology: The magnetic susceptibility of AISI 316L stainless steel was measured on samples produced by selective laser melting (SLM), in the dimensions: (12 x 35 x 3) [mm], layer thickness: 30 [µm], power: 53, 73, 93, 132 [W] and scanning speed: 800, 900, 1000, 1100 [mm/s]; seeking to meet requirements of: adequate surface finish, i.e. low roughness, high density (with low porosity index), according to the standard for metallic materials obtained by additive manufacturing (ASTM F3122-14).
Results and discussion: This occur because there is a microstructural transformation of the austenitic steel surface from the temperature increase generated by the laser beam energy. As the austenitic phase is paramagnetic, but the altered phase is ferromagnetic, a magnetic method was used to identify this transformation. The amount of altered material is tiny, and so the magnetic method must be extremely sensitive. To this end, a device like a susceptibility balance was set up. The use of an analytical balance allowed the measurement of this transformation with acceptable uncertainties.
Conclusions: The powder metallurgy production process using selective laser melting induced the formation of magnetic phases on the surfaces of the evaluated samples, resulting in small but significant changes in the magnetic susceptibility values.

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CONVERSION COATINGS ON PURE MAGNESIUM. CHARACTERIZATION AND CORROSION EVALUATION *IN VITRO*

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Keywords: Temporary implants, magnesium, conversion coatings, in vitro corrosion tests

Introduction and objective: The main limitations for the application of Mg and its alloys as biomedical devices are their high corrosion rate and intense hydrogen evolution in biological media. Conversion coatings are being studied as surface modification route with the aim of controlling magnesium degradation rate. In this work are described the results of a series of conversion surface films obtained on pure magnesium and their characterization. In this initial stage, coated samples were immersed in simulate body fluid solution (SBF) for prolonged periods in order to characterize the degradation process, while electrochemical tests were conducted in the same media.

Methodology: Pure magnesium (99.99 Cordés, Argentina) discs were used after polishing with #400 and #600 abrasive paper. Conversion solution was 40wt% HF. Immersion at room temperature for 5, 25, 50 and 100 hours was evaluated. The samples were studied with scanning electron microscopy, Raman spectroscopy and X-Ray diffraction spectroscopy (DRX). Samples were kept in immersion in simulated biological fluid (SBF) at 37°C for 1, 4 and 6 weeks and then observed the type and extent of degradation and surface damage. Electrochemical tests (corrosion potential, anodic polarization curves and electrochemical impedance spectroscopy) were performed in SBF.

Results and discussion: After immersion of pure Mg specimens in 40wt% HF for 5 hours, an uniform opaque black coating was observed. 25 hours of immersion lead to a more intense and homogeneous black coating, as observed with optical microscopy. After 50 and 100 hours, dark grey surface color coating was observed, with distinctive tones revealing solidification characteristic patterns. No flaws or cracks were detected on the surface coatings with optical microscopy. With SEM microscopy, it resulted evident that all the conversion films covered the surface with an homogeneous layer. The elemental analysis by EDS showed a significant presence of F on the modified surface, and also O in less proportion. The analysis of detailed XPS spectra of Mg, F and O allowed the identification of MgF2 and MgO as components of the conversion layer. However, no evidence of crystalline MgF_2 nor MgO was detected by normal incidence XRD and Raman spectroscopy, then supporting the amorphous structure of the conversion layer.

After 7 days of immersion in SBF at 37°C, all the magnesium specimens present white precipitates and evidence of corrosion damage. However, specimens with conversion treatments evidenced less deterioration than non- treated magnesium. The same trend was observed after 14, 28 and 45 days of immersion. A slight increase in corrosion potential for conversion treatment longer than 24 hours was observed, while longer immersion periods in HF presented lower corrosion potentials than non-treated samples. Additionally, all the magnesium samples evidenced active corrosion during anodic polarization, with lower corrosion densities for 24 hours of conversion, and higher for the coatings obtained at longer periods, compared to non-treated magnesium.

Conclusions: In this work the effect of immersion time in conversion treatments on Mg was studied. Homogeneous films were obtained. 24 hours of immersion in concentrated HF presented promissory results in corrosion tests. MgF_2 and MgO were identified by XPS.

PREPARATION AND CHARACTERIZATION OF TITANIUM-ZIRCONIUM ALLOYS

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Keywords: titanium alloys; zirconium; biomaterials; mechanical properties

Introduction and objective

The alloys used for making dental implants must display properties such as biocompatibility, biofunctionality and corrosion resistance. The titanium alloys are the most widely used due to their higher strength-to-density ratio, superior biocompatibility and corrosion resistance, good mechanical properties, low tensile modulus when compared to other metallic biomaterials. The main objective of this work was the preparation of titanium-zirconium alloys for implant dentistry. The work aimed to compare the results of melting during the various stages of furnace preparation and the corresponding mechanical and microstructural characterization of the obtained titanium-zirconium alloys.

Methodology

A casting of two implant candidate alloys was carried out (Ti14%Zr and Ti20%Zr by mass). The used vacuum melting furnace, an electric arc furnace fitted with tungsten non consumable electrode and billets with 200 g mass were cast. The experimental alloys were chemically characterized using a scanning electron microscope fitted with an energy dispersive spectrometer. The gas analysis was carried out. The microstructure was characterized using optical and scanning electron microscopy. The mechanical properties were assessed using instrumented microhardness testing.

Results and discussion

The resulting melting microstructure observed using optical microscopy, a microstructure in the form of slats or lamellae, also described as basket-weave, can be easily discerned. All microstructures showed the coarse melting microstructures with Widmanstätten lamellae that were related to the inherent anisotropy of the hexagonal crystalline structure of the α phase. With increasing Zr content in Ti-Zr alloy, Widmanstätten lamellar structures became coarser.

Titanium reacts easily with other elements, mainly gases, such as hydrogen, nitrogen and oxygen, dissolving quickly in liquid or solid metal at temperatures above 400 °C, resulting in samples with less ductility or more brittle. The as casted laboratory samples with the addition of 14% zirconium and 20% titanium resulted in increased hardness as compared to commercial sample.

Conclusion

The prepared alloys showed a low carbon contamination, less than 0.03% by mass, indicating that clean melts can be obtained in the electric arc furnace with non-consumable electrode. Samples fused in the laboratory with the addition of 12% and 20% zirconium to titanium caused an increase in microhardness when compared to the commercial sample, which also has about 12% Zr.

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INFLUENCE OF ANNEALING TEMPERATURES ON THE STRUCTURE AND WETTABILITY OF IRON OXIDE NANOTUBES

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Keywords: Biodegradable biomaterials; Iron; Anodic oxidation; Annealing

Introduction and objective: Iron and its alloys are promising as biodegradable medical implants due to their good mechanical properties [1]. Initial studies on pure iron stents show biocompatibility and minimal inflammation but emphasize the need to enhance degradation rates [2]. Surface modification using anodic oxidation, creating nanostructures, could optimize the balance between mechanical performance and biofunctionality in these implants. Therefore, this study aims to enhance a pure iron sample's surface by utilizing anodic oxidation to foster the growth of nanostructures.

Methodology: The technique was performed in an electrolytic solution of ethylene glycol containing 0.3% by mass of ammonium fluoride (NH4F) and 3% by volume of deionized water, under a voltage of 50 V for 30 minutes and agitation at 100 rpm. The anodized nanotubes were annealed in an ambient atmosphere at various temperatures ranging from 150 to 400°C for a fixed period (2h). Morphological and crystalline phase analyses were performed using FE-SEM and XRD. The hydrophilicity of the surface was measured by using the contact angle.

Results and discussion: The nanotubular structure obtained after anodizing and annealing remained stable and uniformly ordered for all samples. In addition, the annealing process transformed the amorphous layer into the magnetite and hematite phases and made the wettability considerably higher, and after annealing at temperatures of 350°C and 400°C the surfaces became super hydrophilic.

Conclusions: Deepening the understanding of the structure and wettability properties of the surfaces examined in this study becomes imperative to advance research and explore the biomedical potential of iron oxide nanostructures.

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HEMATITE SYNTHESIS BY POLYMERIC PRECURSORS METHOD

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Keywords: biomaterials; iron oxide; hematite; Pechini method.

Introduction and objective: Iron is one of the essential nutrients of all microorganisms and acts as a cofactor for several enzymes, is required in metabolic processes, including respiration, DNA synthesis, photosynthetic transport, nitrogen fixation and DNA synthesis [1]. Iron oxide is widely used in biomedical applications due to its physical-chemical properties, its biocompatibility and biodegradability. Hematite (Fe_2O_3) smaller than 100 nm, with a large surface area and chemical stability, has great potential in biomedical applications [2]. The objective of this work was to evaluate which is the best condition for obtaining hematite by the method of polymeric precursors (Pechini method).
Methodology: Iron oxide was synthesized by the polymeric precursor method (Pechini method) and thermally treated to obtain puff. Afterwards, in order to verify the best crystallization temperature of the puff, in which there is pure hematite phase formation, the thermogravimetric (TG) analysis was carried out. After analyzing the results of thermogravimetry, 3 temperatures were evaluated to obtain hematite: 500°C, 550°C, and 600°C. The characterization of the phases formed after the puff treatment was monitored by X-ray diffraction (XRD) and the morphology and grain size of the ceramic powders found were evaluated using the scanning electron microscopy (SEM) technique.
Results and discussion: It can be observed in the thermogram that from approximately 400°C, the decomposition of the ceramic powder is complete, indicating formation of iron oxide phases. Therefore, heat treatments at 500, 550 and 600°C were used, so that it is suitable for the formation of iron oxide. Diffractogram showed the heat treatment at 600°C is the only one with characteristic peaks of single hematite phase. From the micrographs it can be observed that the equivalent diameters of the nanoparticles of the ceramic powders are: 44.65 nm, 95.94 nm and 61.34 nm, respectively. In the micrographs referring to the first and third heat treatment (500°C and 600°C), a rounded appearance of the nanoparticles is observed. In the results of the second thermal treatment (550°C), angular nanoparticles are observed, in which the texture presented is of larger agglomerates, with a larger diameter of the particles. Generally, the higher the heat treatment temperature, the larger the dimensions of the nanoparticles. In this case, the TT at 600°C presented nanoparticles with a smaller diameter compared to the TT at 550°C, probably because this is the temperature at which there is a single hematite phase, unlike the other temperatures studied, where there is a secondary phase.
Conclusions: When analyzing the conditions of the ceramic powder, the crystallization temperature of the iron oxide nanoparticles in which the pure hematite phase is generated was 600°C. At this temperature, homogeneous nanoparticles were formed, which is the most suitable condition biomedical application, compared to heat treatments at 500° and 550°C. Based on X-ray diffraction and scanning electron microscopy, the most suitable calcination temperature for the formation of the pure hematite phase was 600°C.

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POLYVINIYL ALCOHOL MATS CROSSLINKED WITH CITRIC ACID FOR REGENERATIVE MEDICINE APPLICATIONS

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Keywords: nanofibers; biomaterial; papain; polyvinyl alcohol.

Introduction: Currently, biomaterials have stood out in the medical field, seeking innovative solutions for wound healing. Papain, an enzyme with remarkable healing properties, shows promise in this context. However, its activity is compromised when in contact with water and at high temperatures. To overcome this challenge, this project has considered the use of polyvinyl alcohol (PVA) membranes, a low-toxicity, cost-effective and hydrophilic polymer, offering an effective and safe approach to promote wound healing. However, the solubility of this polymer restrains its use, thus the aim of this project is to crosslink PVA fibers directly during solution blow spinning procedure.
Methodology: Mats were produced using a Solution Blow Spinning (SBS) device with a rotary collector. The SBS device was 40 cm away from the collector and the solution was supplied with a pump at 3mL/h through a syringe using air at 6 psi of pressure. Mats were prepared using a 14 wt% PVA (65% high molecular weight/35% low molecular weight) solution in water and compared with solutions containing different concentrations of citric acid. The mechanical and rheological characterizations were done by tensile strength and viscosity tests, while infrared spectroscopy and scanning electron microscopy were employed to analyze fibers structure and morphology.
Results and discussion: The mats produced in this study were consistently white, irrespective of citric acid presence. With increasing citric acid concentration, the mats showed improved resistance, making them more durable for various applications. The addition of citric acid also reduced mats water solubility, enhancing their long-term stability in different environments, notably biological fluids as exudate. Infrared spectroscopy analysis confirmed the crosslinking, while scanning electron microscopy revealed mats were composed by ultrafine fibers. These improved properties obtained by crosslinking make the mats highly suitable for practical applications of wound dressing, mainly by the use of safe crosslinking agent. The PVA solutions presented a higher viscosity with increasing citric acid content, presenting viscosity values of 0.9 ± 0.03 Pa s and 1.19 ± 0.07 Pa s for 0 wt% and 10 wt% of citric acid (in reference to the PVA mass) at 21,5 s^{-1}. These findings hold significant promise for expanding crosslinked PVA mats usage in various types of wounds.
Conclusions: Based on our results, it is possible to assert that with the addition of citric acid, which promoted polymer crosslinking, we obtained PVA dressings with ideal properties for wound use. Since the dressings are in perfect condition to withstand wound moisture, we can now consider the inclusion of papain in the mats, that can be stored dry and hydrated just in the moment of use, an attempt to preserve the hydrolysis of these enzyme.

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TOPIC 3

Biomaterials for tissue engineering and regenerative medicine

COMPOSITE FILMS OF GELLAN GUM, FIBROIN, CARBOXYMETHYL CHITOSAN AND BIOGLASS AS POTENTIAL SKIN WOUND DRESSINGS

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Keywords: Biopolymers, bioglass, wound healing, skin

Introduction and objective: The skin serves as our primary defense against environmental threats. If cases of injury, wound dressings are commonly employed for treatment. There is a growing demand for new wound dressings, with improved properties. Biopolymers and bioactive factors, such as gellan gum (GG), carboxymethyl chitosan (CMCh), fibroin (F), and bioglass (BG) have relevant characteristics as components of wound dressings, e.g. biocompatibility, biodegradability, hydrophilicity, and positive interaction with skin cells. Therefore, the objective of this study was to evaluate the potential utilization of GG-CMCh-F blends, both with and without BG, as wound dressings.

Methodology: Fibroin was extracted from *Bombyx mori* cocoons, solubilized in $CaCl_2$-ethanol-water solution, and dialyzed in water. Aqueous solutions with total polymer concentration of 1% (w/v) were prepared, with mass proportions of 2GG:CMCh:F and 6GG:CMCh:F, and glycerin was added as a plasticizer. The solutions were enriched or not with 1 wt% of BG to improve skin vasculatization and used to produce the films by solvent casting. The films were crosslinked with calcium ions and characterized regarding visual aspect, thickness, surface roughness, contact angle, fluid uptake capacity (24 h), mass loss (7 days) and mechanical properties.

Results and discussion: All films were transparent, facilitating the observation of wound healing progress. The thickness of the films reached at most 60 µm, and since this limit is still lower than that of human skin, the filmswould not be uncomfortable for the patient. All formulations had hydrophilic surfaces, with similar results for roughness (< 1 µm) and water contact angle (< 70°). These results indicate good potential for fibroblast adhesion [1]. Also, the films were capable to absorb water and phosphate buffer saline (PBS) solution in mass ratios above 1.6 and 3.2 after 24h, respectively, ensuring the capability to maintain a hydrated environment and remove excess exudate. The films were relatively stable after exposure for 7 days to water and PBS, showing mass loss values below 35% in these solutions. The films, when wet, showed ultimate tensile strengthswithin the range of 3 to 6 MPa. These values are comparable to commercial dry multi-layered dressings such as Acticoat® and Carbonet™ [2].

Conclusions: The thin transparent films produced showed surface properties suitable for cell interaction. The films are recommended for use on low to moderate exuding wounds, as they demonstrate satisfactory structural stability for 7 days. The mechanical performance of the films is comparable to that of commercial products. Overall, the obtained results demonstrate that the formulations are suitable as wound dressings. However, additional *in vitro* and *in vivo* testing is necessary to further characterize these films and fully assess their applicability.

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ANTIMICROBIAL ANTIBIOTIC-FREE DRESSINGS FOR WOUND HEALING OBTAINED BYPHOTOPOLYMERIZATION

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Keywords: antimicrobial; wound healing; hydrogels; biomaterial

Introduction and objective: Multifunctional wound dressings are currently being developed to address various needs in wound care. However, many of these dressings contain compounds that are potentially toxic or rely on conventional antibiotics, which are becoming less effective against antibiotic-resistant bacteria [1]. This study aimsto synthesize multifunctional skin dressings for wound healing and infection prevention using environmentally friendly techniques, incorporating functionalized silsesquioxanes [2] and natural products. The formulation is designed to cover a wide range of biological functions, including pH response, temperature responsiveness, biocompatibility, antimicrobial properties without conventional antibiotics, and tissue regeneration.

Methodology: To achieve these objectives, hydrogels were synthesized through photopolymerization using cationic and vinylic monomers. Functionalized silsesquioxanes were used as crosslinkers to promote cell regeneration, and natural products were incorporated as antimicrobials. The physical and mechanical properties of the wound dressings were evaluated through tests such as swelling experiments, thermal analysis, and mechanical property measurements. Biological properties were assessed *in vitro* through haemolysis tests, bacterial inhibition kinetics, and viability tests using human cells after wound simulation. Additionally, *in vivo* wound healing experiments were conducted on rabbits.

Results and discussion: The dressings were synthesized in a one-pot photopolymerization step using environmentally friendly manufacturing techniques. The resulting dressings exhibited high swelling properties anddemonstrated pH and temperature responsiveness. They also displayed excellent stability, ductility, and adhesiveness. The biological results showed bactericidal properties with minimal bacterial viability. The dressingswere repeatedly tested with new cultures of bacteria, and after three repetitions, they achieved a 99.99% bacterialkill rate, indicating the intrinsic bactericidal property of the materials and their reusability. Furthermore, the dressings showed low hemolytic effects, high dermal biocompatibility, and significant wound healing effects. These results highlight the potential application of these materials as dermatological wound dressings for woundhealing and disinfection, with reusability properties. All synthesized materials exhibited biocompatibility, antimicrobial properties through the incorporation of natural compounds, and the ability to promote cell regeneration (keratinocytes and fibroblasts). *In vivo* experiments demonstrated that the dressings accelerated wound healing processes by promoting collagen and fibroblast regeneration.

Conclusions: The synthesized dressings exhibited high swellability, pH and temperature responsiveness, haemostatic capacities, bacteriostatic or bactericidal properties, appropriate water vapor transmission rates (WVTR) to prevent dehydration and facilitate fluid exchange, moderate adhesion, and the ability to promote cell proliferation and excellent wound healing, as supported by *in vitro* and *in vivo* experiments. These findings suggestthat these materials have potential applications in the biomedical field as dermatological wound dressings for the healing and disinfection of wounds, offering reusability properties.

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CELL MEMBRANE AS A POTENTIAL CELL-FREE THERAPEUTIC FOR RAPID BONE TISSUE ENGINEERING

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Keywords: cell membrane; bone; biomaterials; cell-free therapeutic

Introduction and objective: Living cell-based therapies have demonstrated promising efficacy in clinical studies but are hindered by several challenges, including logistics, and risks of immunorejection, tumor formation and occlusions in the microvasculature. More recently, emerging evidence has supported cell-free technologies, including cell-secreted extracellular vesicles, as alternative approaches to overcome some of the limitations associated with living cell-based therapies. Previously, we have shown that the nanofragments from the cell (plasma) membrane are nucleation sites initiating bone formation [1, 2]. In this study, we aimed to establish a novel cell-free method using plasma membrane nanofragments (PMNFs) for application in bone tissue engineering.

Methodology: PMNFs were isolated from different cell types (i.e., pre-chondrocytic cell line ATDC5, osteoblastic cell line MC3T3-E1 and NIH-3T3 fibroblasts), lyophilized and transplanted into critical size calvarial defects in ICR mice. Qualitative analysis of PMNF mineralization was performed by electron microscopy, Fourier Transform Infrared Spectroscopy (FTIR) and X-ray diffraction (XRD). Bone repair was evaluated after 2 and 6 weeks by micro-CT and histological and immunostaining analyses.

Results and discussion: The plasma membrane of cells contains numerous receptors, ligands, enzymes and phospholipids that play important roles in cell sensing and signaling, and cell-cell and cell-extracellular matrix interactions. We previously showed that PMNFs are nucleation site for bone formation *in vivo*, and can induce rapid mineralization *in vitro* within 1 day [1, 2]. In this study, we first optimized the methods to obtain PMNFs from different cells and demonstrated that PMNF mineralization initially formed amorphous calcium phosphate (ACP), which subsequently transformed into crystalline apatite. We next applied the pre-incubated PMNFs as a biocomposite material in calvarial bone defects, and demonstrated that PMNFs could repair calvarial bone defects by coordinated and multi-faceted effects on *in situ* cells within the defect. On the other hand, synthesized ACP could also promote bone repair by its osteoconductive properties, but it failed to promote integration with the surrounding naïve bone within the analyzed timeframe. Furthermore, phosphatidylserine, a major component of PMNFs, failed to promote bone repair under the same conditions.

Conclusion: The results of this study support the rationale of utilizing PMNFs as a promising cell-free bone-inducing material having biofunctional properties on *in situ* cells for promoting rapid bone repair.

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FABRICATION AND ASSESSMENT OF FUNCTIONAL POLYCAPROLACTONE/STARCH/CaO SCAFFOLDS FORBONE TISSUE ENGINEERING APPLICATION

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Keywords: electropun scaffolds; polycaprolactone; starch; CaO nanoparticles

Introduction and objective: Bone has a practical capacity for self-healing, but bone defects such as fractures cannot heal spontaneously [1]. Recently, bone tissue engineering (TE) has emerged as an excellent approach to treating injured bone. Electrospun scaffolds obtained from a combination of synthetic (e.g., polycaprolactone (**PCL**)) and natural polymers (e.g., **starch**) have become promising biomaterials due to these are biodegradable, support cell adhesion and proliferation, possess mechanical properties similar to the bone and can mimic the extracellular matrix [1]. Moreover, In order to bring bioactivity, improved osteogenic ability, and increased osteoconductivity properties, **CaO** nanoparticles could be incorporated. This study aimed to design and evaluate functional electrospun PCL/starch/CaO scaffolds.
Methodology: First, a polymeric solution of 15% w/v PCL/starch was prepared in DCM/formic acid, and 5 wt% of CaO nanoparticles (± 16.2 nm) obtained from eggshells were added to the final solution. The solution was electrospun at a constant flow rate of 1 ml/h at 20 kV. Mats were characterized by scanning electron microscopy (SEM). Water absorption and biodegradability were performed by immersing the scaffolds in a phosphate-buffered saline (PBS) solution (pH 7.4). Bioactivity tests were performed to determine the hydroxyapatite (HA) formation by immersion during 28 days in Simulated Body Fluid (SBF). The mechanical properties of the scaffolds were evaluated using the uniaxial tensile test.
Results and discussion: PCL and PCL/Starch and PCL/Starch/CaO scaffolds obtained by electrospinning presented a morphology of randomly arranged fibers with a porous and interconnected structure, demonstrating the high 3D three-dimensional architecture. After incorporating CaO nanoparticles, there was a significant increase in fiber average diameter. A similar result was observed by other authors, who explained that nanoparticles could affect the viscosity and conductivity of the solution during the electrospinning process [2]. The water absorption increased abruptly in the PCL/starch, PCL/CaO, and PCL/starch/CaO scaffolds. The result suggested a hydrophilic material that can more easily lead to cell adhesion and growth. Starch and CaO nanoparticles also increased the degradation of the scaffolds. Young's modulus for PCL/Starch/CaO increased 62% compared with the neat PCL fibers, and this scaffold showed lower elongation a la rupture. The CaO bringsto rigid of the polymer, and this behavior could be due to the CaO nanoparticles dispersed more easily in the starch polymer due to its hydrophilic character. Starch and CaO in the scaffold induced the hydroxyapatite formation on the fiber surface after 28 days of immersion in SBF. This result indicated that the scaffolds are bioactive biomaterials.
Conclusions: Electrospun porous scaffolds of the combination of PCL, starch, and CaO nanoparticles were obtained to develop a bioactive scaffold for bone tissue regeneration. The fiber diameter, mechanical behavior mechanical, water absorption capacity, biodegradation, and hydroxyapatite formation capacity on the surface were strongly influenced by the presence of starch and CaO nanoparticles. Therefore, results confirmed that these biomaterials possess the potential for bone tissue regeneration.

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BIOLOGICAL EVALUATION OF ELECTROSPUN FIBERS BASED ON POLYACRYLONITRILE/CALCIUM OXIDE NANOCOMPOSITES AS A BIOACTIVE SCAFFOLD FOR BONE TISSUE ENGINEERING

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Keywords: Polyacrylonitrile scaffolds, calcium oxide nanoparticles, bone tissue engineering, bioactive biomaterials

Introduction and objective: Electrospinning is a simple, versatile, and cost-effective technique that allow to obtain a 3-D porous structure based on polymer fibers with nano or micrometric size mimicking the extracellular matrix (ECM) [1]. Polyacrylonitrile (PAN) is a commercial polymer with good mechanical and chemical stability, and with good biological behavior becomes an interesting polymer material for biomedical applications [2]
The aim of this work is developing a functional scaffold based on Polyacrylonitrile (PAN) reinforced with calcium oxide nanoparticles obtained from clam shell waste and evaluate their biological properties as a potential scaffold for bone tissue engineering.
Methodology: PAN and PAN/n-CaO fibers with 2.5, 5, 10 and 20 wt.% were developed by the electrospinning technique, the solutions were prepared at 6% w/v in Dimethylformamide (DMF). The various amount of n-CaO were added into Flask with 5 mL of DMF and sonicated for 1 hour. PAN was added and the total volume of DMFis completed and allowed to stirr for 24 hours. The electrospinning process were carried out using a voltage of 20 KV and a flow of 1.6 mL/hour at room temperature. The fiber mats are collected, labeled and storage under refrigeration.
Results and discussion: PAN/n-CaO fibers had a randomly deposition with a good homogeneity and interconnected pore structure. The incorporation of CaO decrease the fiber diameter and decrease the hydrophobicity compared to neat PAN. Furthermore, the PAN/CaO shown a bioactivity ability after 21 days ofimmersion in SBF solution and for other side, after in vitro analysis using a human fetal osteoblast HFOB-1.19cell line, the fiber mats did not show cell cytotoxicity allowing the cell adhesion and proliferation in the surface.**Conclusions:** The develop of electrospun mats based on PAN matrix with the incorporation of calcium oxide nanoparticles are an interesting route to prepare a bioactive scaffold with a good biological behavior being a suitable material for bone tissue engineering applications

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PLA-BioAlSr GLASS COMPOSITE AS A CANDIDATE FOR BONE AND TENDINOUS REGENERATIONAPPLICATIONS

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Keywords: biomaterials; tissue engineering; poly(lactic acid); bioactive glasses

Introduction and objective: Poly (lactic acid) (PLA) is currently one of the most promising biodegradable and biocompatible materials once it can be produced from renewable resources and used in packaging, biomedical and tissue engineering applications. In the past decade, composite materials combining biodegradable polymers with inorganic materials such as bioactive glasses are being studied showing remarkable improvements mostly in mechanical and thermal properties. Previous studies conducted by our research groupproposed a bioactive glass composition containing strontium and alumina, BioAlSr, ensuring osteointegration and superior mechanical properties [1]. Here, a composite film of PLA and BioAlSr was proposed for bone tissue regeneration.

Methodology: To produce the composite films chloroform was used cast solvent. The PLA was dissolved in chloroform at 3,3g/ml and the BioAlSr particles was added in ratios of 15, 30, 50 and 70 wt%. The glass particles were submitted to ultrasonic treatment to permit more efficient dispersion in the PLA matrix. The obtained films were analysed for their microstructure with X-ray diffraction and Fourier transform infrared analysis, homogeneity by polarized light microscopy and and in vitro cytotoxicity by NCTC clone 929 cell line.

Results and discussion: All XRD spectra showed predominantly amorphous state of the composite films. To examine the existence and type of interfacial interaction in the composites, FT-IR experiments were performed and compared with pure PLA and BioAlSr. The regions of interest were 1780 and 1680 cm^{-1} for the C=O stretch, and 3600–3000 cm^{-1} for the O–H stretch from PLA known signatures [2] and the region of 800-1300 cm^{-1} corresponded to the stretching vibrations of the silica of the bioactive glass was analysed [1]. PLA characteristicbands were predominant even in samples containing 50 and 70 wt% of BioAlSr. The dispersive effect of the glass particles in the PLA matrix was also evaluated by polarized light microscopy and the results demonstrated an adequate homogenization. Also, cytotoxicity and cell viability obtained by using the NCTC clone 929 cell line did not show any significant loss of cell viability or cytotoxicity.

Conclusions: Preliminary results of the proposed study indicate the obtention of a homogeneous composite film with adequate interaction between the matrix and the dispersed material, preserving the microstructure of both materials. Furthermore, the material obtained did not show cytotoxicity, indicating that it is a promising alternative for the application of bone and tendinous regeneration.

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PRODUCTION OF BIONANOCOMPOSITES OF CARBON AND DIETARY PROTEINS AS APROMISING DRESSING MODULATOR OF INFLAMMATION IN BONE REPAIR

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Keywords: Scaffold; Oral tolerance; Bone repair; Inflammation

Introduction and objective: Scaffolds provide an ideal cellular environment for differentiation, proliferation, and division. Collagen-based scaffolds are biocompatible and, when associated with carbon-based materials, show significant potential for inducing osteogenesis [1]. Recent studies on skin wound repair in mice and bone injuriesin rats have revealed that the parenteral injection of previously ingested proteins, such as zein, promotes tissuehealing improvement by modulating the inflammatory process, reducing the production of specific antibodies, and increasing T lymphocytes [2]. This work aims to develop a new biomaterial composed of type I collagen associated with a carbon-based material functionalized with the zein protein to enhance bone injury repair. **Methodology:** Multi-walled carbon nanotubes (MWCNTs) were functionalized with zein protein (Sigma-Aldrich).Bovine tendon collagen was extracted and associated in different concentrations with the nanomaterial to produce the dressing. For characterization of the produced materials, analyses were performed using Fourier- transform infrared spectroscopy (FTIR), Raman spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), and atomic force microscopy (AFM). *In vitro* tests of ALP, viability, and biomineralization wereconducted with primary neonatal Wistar rat osteoblasts. **Results and discussion:** The FTIR and Raman spectroscopy techniques indicated successful functionalization of MWNTCs with zein and their combination with collagen at 2 mg/ml, as evidenced by the appearance of new peaks corresponding to newchemical groups observed in the graphs. The topographical morphology by SEM demonstrated that the porosityof collagen scaffolds is not significantly affected by the association of functionalized or non-functionalized nanomaterials with zein. The average pore diameter of the different produced groups varied between 143 µm to 178 µm, with the ideal size for the migration of bone cells being ≥ 100 µm, suggesting that the produced material is suitable for bone repair tests. AFM showed better mechanical resistance in scaffolds with 2% MWCNTs, suggesting this as the ideal concentration for biomaterial production. The *in vitro* tests conducted on primary osteoblast cultures demonstrated that the cells remained viable and metabolically active in all tested concentrations of MWCNTs. **Conclusions:** Therefore, the present study achieved success in producing a cytocompatible biocurative, with promising results that generate a perspective of excellent outcomes in future in vivo studies.

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NANOGEL-BASED ADVANCED THERAPEUTIC FOR NOSE-TO-BRAIN DELIVERY TO TACKLE OXIDATIVE STRESS

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Keywords: Single Enzyme Nanogel, Catalase, Neurodegeneration, Nose-To-Brain

Introduction and objective: Oxidative stress is linked to aging and neurological disorders. Among them, MultipleSclerosis (MS), frequently diagnosed in young adults, is the main non-traumatic cause of disability in young adult.[1] With no available cure nowadays, most injectable or oral drugs can only slowdown disease progress. Hence, since Blood Brain Barrier (BBB) remains intact in MS, the systemic administration route is highlyinefficient. Thus, we aim to develop Single Enzyme Nanogels (SEN) that efficiently delivers antioxidant enzymes (using catalase as a model drug) into the brain exploiting the nose-to-brain route, which would represent a disease-modifying therapy for MS as an alternative to traditional formulations.

Methodology: SENs, are gathered by using monomers and crosslinkers surrounding each individual enzyme by free radical polymerization.[2] All the systems are characterized in depth in terms of size, surface morphology, chemical composition and z-potential, by SEM, TEM, FPLC, FT-IR and DLS measurements. In order to test the transport ability of the designed systems, a transfer model of Calu-3 is implemented. Finally, cell viability using fluorescence-based alamarBlue™, PicoGreen™, and the phenotype of the cultures is assessed by ELISA by measuring expression of markers of inflammation and oxidative stress.

Results and discussion: A screening of SENs with different surface properties was successfully fabricated in a reproducible way by using different monomers (APTAC, (3-Acrylamidopropyl)trimethylammonium chloride; MAEP, (Monoacryloxy)ethyl phosphate; DMAPS, [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide) at an optimized ratio. The SENs present zeta potential of +14 (APTAC), -22 (MAEP) and -14 mV (DMAPS) respectively, proving the incorporation of the monomers in the surface of catalase (with a zeta potentialof -7 mV). Moreover, these values were stable over time. Overall, this data showed that the SENs presented good physicochemical properties to protect the free catalase in the biological milieu. The alamarBlue® and PicoGreen® assays assessed the mitochondrial activity and DNA content of macrophages (THP-1) and microglial (HMC-3) cell lines. No significant differences between untreated and treated cells were achieved. The inflammatory profile ofthe THP-1 and HMC-3 cells after the treatment was assessed by ELISA of the most relevant markers (TNF-α, IL- 1β and Il-6), showing an improvement compared with controls. The permeation through the air epithelium was also tested by using a Calu-3 based transwell model, showing potential for its nose-to-brain delivery.

Conclusions: SENs carrying catalase were successfully fabricated and exhibited potentially good physicochemicalproperties for its nose-to-brain delivery. Moreover, they exhibited a good performance over model cell lines of inflammation and oxidative stress. In a second phase, SENs will be carried into Mucoadhesive Nanogel (MNG) which would made the network biodegradable and provide an increased high adhesion potential. Hence, this willimproved the mucopenetration capabilities of the systems in order to cross the nasal epithelium to reach the brain in an efficient manner.

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BIOGEL WITH PIEZOELECTRIC NANOPARTICLES AS OSTEOINDUCTIVE BIOMATERIAL

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Keywords: Biomaterial; Barium titanate; Osteoblast; Maturation, Piezoelectric.

Introduction and objective: Osteoblasts produce mainly collagen type I, in association with calcium and phosphate ions organized in the form of hydroxyapatite crystals, constituting an ECM that gives the tissue piezoelectricity, understood as the ability to transform mechanical stimuli into electrical signals, which have thepotential to influence cellular activity and consequent bone remodeling. Therapeutic strategies that mimic the physical properties of bone are of great relevance in the treatment of various injuries. Thus, the aim of this study was to produce and evaluate the osteoinductive potential of collagen biogels with piezoelectric barium titanatenanoparticles (NPTB).

Methodology: The samples (collagen and collagen with NPTB groups) were analyzed by SEM, Electric Force Microscopy, Raman, FTIR, zeta potential, dynamic light scattering, and DRX characterization methods. In vitro assays were performed using osteoblasts from the calvary of neonatal Wistar rats. Cytocompatibility tests wereperformed by alamar blue assay, and ALP activity and quantitative PCR were evaluated. We used adult Wistar rats underwent surgery to create a bone defect of 2 mm in diameter in the proximal middle third of the tibia andwere treated with biomaterials. After 14 days post-surgery the bones were evaluated by histomorphometric analysis.

Results and discussion: The cells were exposed to different concentrations of NPTB, for which no cytotoxicity was identified. When associated with the collagen gel extracted from the rat biogel at a final concentration of 2mg/ml, the NPTB at a concentration of 1% (w/v) formed a cytocompatible scaffold on which the cells showed typical morphology of osteoblasts with cytoplasmic processes in contact with the biogel surface. Furthermore, rat collagen biogels with NPTB were able to promote osteoblastic maturation observed after 14 days of cell culture through the increased activity of the enzyme alkaline phosphatase, involved in the process of bone mineralization, for which a statistically significant difference was identified ($p< 0.05$) in relation to the control group in which the cells were cultured on a plastic surface. Moreover, in the presence of collagen associated with NPTB there was significant expression of genes related to osteoblastic maturation, such as Col1a1 ($p<0.005$), Alpland Bglap ($p<0.0001$) when compared to the control, demonstrating the potential of such nanoparticles as osteoinductive material. The in vivo assay showed a significant increase in new bone formation area in the collagen biogel with NPTB.

Conclusions: Thus, the biogel developed is a cytocompatible biomaterial that mimics physical and chemical properties of bone tissue with the ability to induce maturation of osteoblastic lineage cells, and improve bone repair in vivo, presenting potential for application in future strategies of bone regeneration.

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ELASTIN LIKE HYDROGELS FOR BRAIN REPAIR AFTER ICHEMIC STROKE

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Keywords: Hydrogel, Extracellular Matrix, Elastin, Stroke

Introduction and objective: To date, there is no efficient treatment for ischemic stroke (IS). Our brain cannot self-regenerate. Inflammation, neurogenesis, and angiogenesis are the key players when it comes to post-stroke key changes in the brain. The inflammatory response results in a cystic encephalomalacia, lately referred as “stroke cavity” within biomaterial science. This is the therapeutic window in which post-strokebrain regeneration assisted by biomaterials stands [1]. Extracellular Matrix (ECM)-like hydrogels can be safely implanted to promote endogenous repair mechanisms and achieve long term recovery after stroke [2]. Here, westudied the therapeutic post-stroke effect of elastin-like hydrogels in vitro and in vivo.

Methodology: Elastin-like, catalyst-free, in situ forming, “click” hydrogels (ECFCGs) were synthesized by crosslinking two specific elastin like recombinamers through Huigsen 1,3 dipolar cycloaddition. The physicochemical properties of the ECFCGs were fully characterized. Primary mixed glial cultures (PMCGs) from isolated cells from Sprague Dawley rat brains were used to study the ECFCGs *in vitro*. Particularly, an optimized Oxygen and Glucose Deprivation (OGD) model was implemented to emulate ischemic conditions *in vitro* to test the ECFCGs therapeutic effect. Finally, a rat Middle Cerebral Artery Occlusion (MCAO) model was used to study the ECFCGs effect on functional recovery *in vivo*.

Results and discussion: ECFCGs physicochemical properties are suitable for the intended application and implantation route. *In vitro*, ELR induced significant increase in cell proliferation in healthy and OGD-exposed PMCGs. Furthermore, an antinflammatory effect in OGD- exposed PMCGs. *In vitro* and *in vivo* an anti-inflammatory effect was observed after ECFCGs treatment. Moreover, *in vivo*, neurobehavioral tests suggest a restored motor function and amelioration of overall brain damage (data still being processed). The results indicate that the biomaterial proposed provide an amenable microenvironment for cell infiltration, angiogenesis, neurogenesis in and around the stroke lesion and, a timely in situ degradation due to the overexpression of post-stroke MMP-9 and an anti-inflammatory response in terms of a reduction in microgliosis and astrogliosisis probably (ongoing analysis) related with a reduced scar thickness and decreased reactive microglia. This work develop, fabricate in vitro and preclinically evaluate for the first time elastin- inspired in situ-forming bioactive hydrogels to fulfil an unmet clinical need.

Coclusions: ELCFCGs are suitable biomaterials to address IS severe outcomes. These hydrogels are showing neuroprotective effects in a manner linked to the modulation of the critical elements of IS pathophysiology,likely preserving the perilesional non-necrotic area.

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USE OF ALGINATE HYDROGEL TO IMPROVE GAIT IN ANIMALS WITH OSTEOARTHRITIS

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Keywords: biomaterial; hydrogel; osteoarthritis; gait test;

Introduction and objective: Osteoarthritis (OA) is an inflammatory disease of the joints, like the knee, it causesgradual wear and tear of articular cartilage. As the global population ages cases of OA rise and impact on joint function and mobility, necessitating effective treatment approaches [1]. The aim of this study is analyzing the efficiency of alginate hydrogels loaded with anti-inflammatory compounds extracted from marine sponges in the march of the animals.

Methodology: 12 male ***Rattus norvegicus albinus,*** at 11 weeks old were OA induced by anterior cruciate ligament transection. The alginate hydrogel was applied twice after the induction, 28 and 45 days. The gait tests were conducted on a control and treatment group, the animals had hind paws marked and walked on a track, measuring 60 cm long by 9.5 cm wide. The footprints were digitized, and 4 variables were taken[1]:

- Paw length
- Paw width
- Stride length
- Paw area

On the 60th day after the OA induction they were euthanized. Statistical test was performed using two-way ANOVA.

Results: No statistical differences were found between the different groups in terms of paw length, paw width, stride length and paw area, both at the beginning and at the end of treatment (p= 0.3113, 0.73, 0.582, 0.0527, respectively). It was also not possible to detect differences in the same group at the different moments evaluated in the variables after induction and after treatment, for the control group (p=0.582, 0.0492 0.0592, 0.0569, respectively). This abstract is part of the partial results of the study and the low number of animals evaluated so far may have interfered with this lack of statistical difference.

Conclusions: We can relate these results to a low experimental number, the experiments are still occurring. With the accomplishment of this study it was possible to verify that the alginate hydrogel enriched with satin-inflammatory compound extracted from the marine sponge was not efficient to improve the gait of animals with OA.

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BIOLOGICAL EVALUATION OF BIODEGRADABLE POLYMER (PLA/β-TCP) IMPLANTS FORTHEIR POTENTIAL APPLICATION IN BONE TISSUE ENGINEERING

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Keywords: polylactic acid; β-tricalcium phosphate; bone regeneration; experimental animal models

Introduction and objective: Biodegradable polymers are an alternative material for bone defect rehabilitation. CONICET-INTI developed a polylactic acid polymeric (PLA) biomaterial reinforced with β- tricalcium phosphate (**β-TCP**) at different concentrations (2.5 and 5%) [1]. The PLA/β-TCP-2.5% showed better mechanical properties as a potential biomaterial for bone tissue engineering. *In vivo* studies are therefore needed to evaluate biological response to the biomaterial and determine its efficacy as a bone substitute. The aim of this study was to evaluate tissue response to this novel biodegradable polymeric biomaterial (**PLA/β-TCP-2.5%**) developed in our country, at one and six months post-implantation using two experimental murine models. **Methodology:** Compact **PLA/β-TCP-2.5%** implants were placed in the subcutaneous cellular tissue (group SCG) and bone marrow of the tibia (group BMG) of 20 male Wistar rats [2]. The animals were euthanized one and six months post-implantation, and tissue-implant samples were obtained. The samples were fixed, demineralized, embedded in paraffin, and stained with H-E for histological examination to determine the presence of inflammatory infiltrate, signs of biodegradation, and multinucleated giant cells (MNGCs) with and without material in their cytoplasm; MNGCs containing particles were analyzed by SEM-EDS. BMG samples were also analyzed histomorphometrically to evaluate new bone formation ($p<0.05$). **Results and discussion:** Histologically, SCG samples showed fibrovascular tissue on the periphery of the biomaterial and MNGCs containing particulate material, indicative of the onset of biodegradation, one and six months post-implantation. EDS analysis of the intracellular particles revealed the presence of oxygen and carbon, corresponding to the PLA. The number of MNGCs increased significantly through time ($p<0.05$). BMG samples showed areas of woven bone in contact with the surface of the biomaterial with no inflammatory response and fibrovascular tissue with MNGCs penetrating the surface of the biomaterial, indicating the onset of biodegradation, one month post-implantation. Six months post-implantation, they exhibited lamellar bone in close contact with the implant and penetrating its surface, with no related inflammatory infiltrate. Histomorphometric determinations showed 79.2 ± 2.1% of lamellarbone in contact with the implant surface. Slow degradation at the surface of the biomaterial and an increase in the number of MNGCs were observed through time. Although the woven bone was replaced with lamellar bone, which is biomechanically suitable to withstand forces, new bone formation was only observed at the surface. **Conclusions:** The compact biodegradable polymeric biomaterial (**PLA/β-TCP-2.5%**) studied here showed adequate biocompatibility and was found to promote new lamellar bone formation six months post-implantation. Nevertheless, the occurrence of biodegradation at the surface only and lack of tissue penetration into the core of the implant show it is necessary to modify the overall 3D structure and architecture of the implant to make it less compact and favor biodegradation and tissue penetration.

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IN VITRO AND *IN VIVO* COMPATIBILITY OF ELASTIN-LIKE RECOMBINAMER-BASED HYDROGELS

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Keywords: elastin; recombinant protein; bone; scaffold

Introduction and objective: Advances in recombinant DNA-mediated protein production allow the production of new "tailor-made" proteins, or protein combinations with potential applications in tissue engineering. We have developed a biomaterial hydrogel based on elastin-like recombinamers (ELR) using recombinant genetic technology that allows the introduction of some specific bioactive sequences to improve its functionality. Our aim was to investigate their quality and whether our ELR-based hydrogel was biocompatible and regenerated bone tissue.
Methodology: Two different ELRs were developed, RGD cell adhesion sequences and metalloproteinase sensitive sequences. Both were cloned into a vector for expression in *E. coli*. They were then modified to contain azide and cyclo-octino groups. Later, the two ELRs were mixed together to form a cross-linked hydrogel. We performed cell viability tests by culturing encapsulated hydrogels fibroblasts using the LIVED/DEAD kit. 12 rabbits were assigned to control or injury, with hydrogel on the left side and no hydrogel on the right side. Clinical controls and biochemical were done at 7, 15, 30 and 90 days, as well as tomographic studies at day 90 and post-mortemhistopathological studies.
Results and discussion: Cell viability was 99%. There were no differences in clinical or biochemical studies between the control and injured groups. Tomographic studies of left injuries showed mineralized tissue compatible with bone tissue, while right injuries showed small diffuse areas with increased density. Histopathological studies of left injuries evidenced formation of multiple reticular bone islands with a neoformation similar to intramembranous ossification, whereas right injuries showed fibroblastic-fibrous tissue.Our hydrogel evidenced high cell viability, biocompatibility and ability to promote regeneration in an *in vivo* model. This showed the potential of this hydrogel for bone tissue regeneration. Our in vivo model demonstrated that the angiogenesis occurs to promote proliferation, migration and angiogenic neovascularization, allowing the colonization of osteoblastic cells in the hydrogel area.
Conclusions: It is therefore concluded that excellent results have been achieved using a bicomponent hydrogel composed exclusively of ELRs, on the premise that generating an optimal cellular environment is the most effective way for the cells surrounding the lesion to fully develop their regenerative capacity; it is easy to apply in situ; it does not require the inclusion of a bioceramic phase; and it will present less regulatory complexity since it does not involve cells in the therapeutic process.

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EFFECT OF ZINC IN THE PROCESS OF HIGH ENERGY MILLING OF POWDERS TO OBTAIN THE ALLOY OF THE Mg-Zn SYSTEM AIMING AT BIOMEDICAL APPLICATIONS

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Keywords: Zinc; Mg-Zn; High energy grinding; biomaterials.

Introduction and objective: The research develops alloys of the Mg-Zn system with potential biomedical application, bioresorbable and bioactive, with good interaction with the organism. Zn in these alloys plays a crucial role, stimulating bone formation and inhibiting bone resorption, making it a suitable element for Mg biomaterials, as well as having a modulus of elasticity, favoring osseointegration[1]. Thus, the objective of the research is to use the grinding to obtain alloy powders from the Mg-Zn system, improving the concentration distribution of the elements, with Zn present as a precipitating agent in Mg alloys, being one of the main alloying elements used in Mg [2].

Methodology: The high energy grinding was used for reduction, uniformization and homogenization of the systemparticles. The powders produced were characterized to monitor the milling process. Subsequently, the powders mixed, subjected to conformation by pressing and synthesized. The microstructural changes during the process were verified regarding the formation of the stoichiometry of interest, highlighting the Zn in the alloys of the Mg- Zn system. The compacted and sintered samples were characterized for microstructure and mechanical properties by analysis by (XRF), (XRD), (SEM+EDS), compression assay and microhardness.

Results and discussion: The results obtained with the experiments carried out and the activities developed in thisstudy of the alloys of the Mg-Zn system, are presented and discussed following the experimental methodology and analyses presented. Zinc is an important alloying element in the Mg-Zn system, as it allowed to increaseductility, deformability, tensile strength and alloy hardness in this system. where also decrease the content in impurities. It was found that the microstructure of the alloys of the Mg-Zn system strongly depends on the Zncontent. In obtaining the powder of the alloys of the system under study the high energy grinding process refinedwith the increase of the % in weight of Zn, significantly the size of the particles of the elements reached differentsizes, with some phase transformations present in the powder before sintering, confirmed in the analysis of XRD,SEM + EDS and calculated by the Thermo-Calc software, presented relevant functionalities along its size scales. **Conclusions:** The Zn in the Mg alloys played a significant role in the refinement of the grains duringsintering. However, due to the compact hexagonal crystal structure (HCP) of Mg and Zn, the low ductilityof Mg resulted in the lack of active sliding systems at room temperature. This made Mg alloys sensitiveto grain size, with smaller sizes leading to higher strengths, particularly in the Mg2Zn system alloys in thisstudy.

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HIGH STRENGHT INJECTABLE PRE-MIXED SILK FIBROIN/α-TRICALCIUM PHOSPHATEBONE CEMENT

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Keywords: tricalcium phosphate; silk fibroin; pre-mixed bone cement; biomaterial.

Introduction and objective: α-TCP-based cements have attracted interest as potential solutions for bone filling and regeneration, owing to their favorable biological properties and their applicability in minimally invasive procedures [1]. Efforts have been made to improve the handling and the resulting statistical deviations in the cement's properties through the development of pre-mixed and dual-paste formulations, which incorporate additives that hinder the hydration reaction of the α-TCP [2]. However, the mechanical properties of injectable α-TCP cements remain inadequate for numerous orthopedic applications. Therefore, the main objective of this research is to develop an injectable pre-mixed α-TCP cement reinforced with silk fibroin solutions.
Methodology: Pre-mixed CPC pastes were prepared by the mixture of a synthesized α-TCP powder with a silk fibroin/$MgCl_2$ solution, in order to obtain a storable paste. This procedure was performed for solutions with different SF concentrations and the pastes were stored for 7d in hermetically sealed syringes. The α-TCP hydration reaction was activated by adding a $CaCl_2$ solution to the pastes. The mixing was carried out by attachingtwo syringes and pumping the pastes between the syringes. The developed CPCs were characterized by XRD, XRF and FTIR, and had their injectability, porosity, compressive strength, microstructure and behavior in SBF assessed.
Results and discussion: CPCs with SF reinforcement exhibit enhanced efficiency in the α-TCP hydration reaction.In addition to the common transformation of α-TCP to CDHA, the presence of chloride ions in the inhibition andactivation solutions induced the formation of small concentration of chloride-substituted hydroxyapatite (HAp- Cl). While the CPCs injectability slightly decreased with higher SF concentrations, the injectability for the CPCs with better mechanical properties remained suitable for application. Mechanically, the SF-reinforced samples demonstrated a significant improvement in compressive strength. For instance, samples with 0.34wt% SF show 314% increase in compressive strength, reaching 40.73MPa, despite having porosity in the order of 55%. Moreover, the microstructure of SF-reinforced samples exhibit biomimetic features, with smaller needle-like CaP crystals, which potentially contributed to the material's reinforcement. Furthermore, the reinforced pre-mixed cements display increased apatite deposition and minor changes in the SBF solution pH compared to the controlsample. And, when evaluating the capacity of the material to be applied as an injectable cement, the paste, wheninjected in SBF, notably maintained its cohesion, exhibiting minimal washout behavior and maintaining its mechanical properties.
Conclusions: This research successfully obtained a high-strength injectable pre-mixed CPC through the addition of SF. The obtained properties demonstrate an improvement in mechanical strength and in the washout behaviorof the paste, while maintaining its injectability. The microstructure demonstrated the capacity of SF to regulatethe CaP crystal's growth, assisting in the higher apatite deposition, which could infer a higher bioactivity. The reinforced CPC is, therefore, a material with potential to be further studied as a suitable material for bone regeneration.

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POTENTIAL IODOPHOR-BASED WOUND DRESSINGS VIA SOLUTION BLOW SPINNING

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Keywords: polycaprolactone (PCL); polyethylene glycol (PEG); antimicrobial agent; fibrous material

Introduction and objective: Due to the increase in life expectancy and the aging of the population, as well as the rise of obese, diabetic, and cardiovascular patients, the number of individuals affected by chronic wounds iscontinuously growing. Aiming to contribute to these injuries' treatment, this study focused on developing a potential antimicrobial dressing using the solution blow spinning technique (SBS). Therefore, iodine, as an antimicrobial agent used for over 150 years, was loaded into spun mats of polycaprolactone (PCL), a biocompatible and biodegradable hydrophobic polyester, containing or not polyethylene glycol (PEG), a biocompatible polyether with high water absorption capacity [1].

Methodology: 12% (m/v) PCL was solubilized in glacial acetic acid under constant stirring at room temperature for 3 h. A second solution was prepared by adding 1% (m/v) PEG to the PCL solution. Lugol's (iodine/potassium iodide aqueous) solution was added to this mixture based on the concentration determined by the minimum inhibitory concentration (MIC) assay against *S. aureus*. Mats were spun using SBS equipment at a flow rate of 6 mL/h, air pressure of 10 psi, and a working distance of 30 cm from the target. The morphology, physicochemical properties, and in vitro antimicrobial activity of produced mats were assessed.

Results and discussion: Scanning electron microscopy (SEM) analysis enabled the observation of fiber and
bead presence in the morphology of all studied mats and the quantification of these structures. PCL mat exhibited fibers and beads measuring 570 nm and 11.34 µm, while PCL/PEG mats, 470 nm, and 10.43 µm, respectively. The addition of PEG imparted hydrophilicity to the PCL mat and increased its swelling capacity, potentially improving its ability to absorb exudate at the wound site. Based on the MIC assay against *S. aureus*, Lugol's solution was added to the polymer solutions at concentrations of 2.84 and 4.26 mg/mL to produce iodine-loaded mats. From UV-Vis analysis, it is hypothesized that iodine ions and oxygen atoms from the polymer chains of PCL and PEG formed a charge transfer complex, potentially generating iodophor materials and making iodine release safer for human cells [2]. Furthermore, the presence of PEG on the mats enabled a higher rate of iodine release within the first 24 hours, while antimicrobial assays demonstrated that PCL/PEG mats with 4.26 mg/mL of iodine were the most effective against gram-positive and gram-negative bacteria. Scratch tests showed that PCL/PEG/iodine mats exhibited *in vitro* wound healing potential.

Conclusions: Based on the results obtained, it is possible to conclude that the iodine provided the desired antimicrobial characteristics to the material. Moreover, PEG, besides aiding in the faster release of the antimicrobial agent, provided the potential for the absorption of wound exudate by the spun mats. Based on these results, it can be inferred that the iodophor-loaded PCL/PEG mats have potential for application as modern wound dressing.

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ROTARY JET SPINNING – A VERSATILE TECHNIQUE: SCAFFOLDS OF PCLWITH BIOCERAMICS OR PHYTOTHERAPICS FOR TISSUE ENGINEERING

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Keywords: biocompatible materials; bioactive molecules; biomaterials.

Introduction and objective: Rotary jet-spinning (RJS) presents as a facile, cost-effective, and efficient method with a high fiber production, utilizing high-speed rotating polymer solution jets to extrude fibers that can range nano to micro scale, depending mainly on solvent evaporation and speed. Policaprolactone (PCL) scaffolds haveshown efficacy in the osteogenic differentiation of mesenchymal cells, proving their performance as an osteogenic biomaterial. Calcium phosphates stimulate the growth of new bone, being soluble and other bioactive molecules that can modulate the inflammatory response, as Resveratrol (RESV). In the present work the proposalwas to obtain PCL scaffolds with □-TCP or RESV for tissue engineering.
Methodology: Scaffolds of PCL/β-TCP were produced with 5 and 10 wt% of bioceramics, being initially dispersedin chloroform, followed by polymer addition at 18.75% w/v. PCL/RESV samples were obtained with PCL dilution in chloroform with constant magnetic stirring for 60 min and RESV dilution was performed in acetone for 7min in a flask protected from light. After complete dissolution of RESV, its solution was slowly poured into the polymeric solution (PCL) and, after complete mixed under magnetic stirring. The solutions were slowly dispersedin a tank and submitted to 3,500 rpm constant rotation for fibers production by rotary jet-spinning process.
Results and discussion: Scaffolds obtained in this study presented the pattern of random distribution of fibers formed in all samples and porosity, an additional factor that benefits cell adhesion in polymeric scaffold, indicating that they could be used as bone grafting procedures around implants or even as filler material in inlay graft techniques for alveolar bone augmentation, in which high mechanical strength is not necessary. Beta- tricalcium phosphate is one of the most efficient bioceramics for bone reconstitution showing good biocompatibility, reproductibility, non-immunogenicity and, has a chemical composition close to the mineral phase of the bone. RESV was used with the purpose that, when implanted, the scaffolds can release the phytotherapic as it is gradually released in situ during the PCL resorption process, that is, the scaffolds serve as implants aimed at RESV drug delivery in the surgical bed. During cell culture and viability tests, both samples were classified as nontoxic, providing adhesion and cell growth. Ultraviolet-visible spectroscopy of the PCL/RESVsamples, showed deviation in the absorbance curves, suggesting the release of RESV in the analyzed solutions over time.
Conclusions: Polymeric scaffolds of PCL/β-TCP and PCL/RESV were successfully produced by rotary jet spinning process, presenting the morphology desired in biomaterials for use in bone regeneration, consisting of microfibers and micropores that can promote cell adhesion and proliferation. These biomaterials stand out for their bioactivity and grafting flexibility; however, still demand additional novel clinical studies.

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POLYCAPROLACTONE TRIOL-BASED POLYURETHANE FILM EMBEDDED WITH CITRUSOILY: ANTIBACTERIAL AND UV-BLOCKER PROPERTIES

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Keywords: PCL T, Polyurethane, Terpenes, Wound dressing

Introduction and objective: Polyurethanes (PU) are among the most ubiquitous materials in the biomedical and pharmaceutical areas. Limonene is one of the most abundant terpenes in nature and is commonly found in thepeel of lemons, oranges and tangerine. The research was carried out with the aim of evaluating the incorporation of extracts from citrus fruit peels (lemon, orange and tangerine) in PU films for topical dressings. Limonene exhibits the ability to disrupt and penetrate the lipidic structure of the bacterial cell wall, leading to protein denaturation and destruction of the cell membrane [1].
Methodology: Polyurethane (PU) and citrus-PU (orange, lemon and tangerine) films were synthesized followinga one-step condensation protocol using polycaprolactone triol (900 $g.mol^{-1}$) and 1,6-hexamethylene diisocyanate [2], and characterized according to composition (FTIR), morphological (MEV), thermal (TGA), mechanical, swelling, antibacterial, and also the amount of resveratrol (RSV) was evaluated by an Agilente high-performance liquid chromatograph (model 1200 Infinity). In addition to hemocompatibility and cytotoxicity to PU.
Results and discussion: The synthesized PU film had a smoother surface and superior mechanical properties compared to PU-citrus films, and the incorporation of citrus fruit peel extracts into PU films reduced the modulus of elasticity and increased the strain at break. Orange (Ora-PU), lemon (Lem-PU) and tangerine (Tan-PU) films showed that they are thermally stable at temperatures up to 250 °C. Moreover, PU and PU-citrus films were capable of swelling in PBS while PU-citrus films followed by weight loss. The PU film not only had excellent hemocompatibility, but also increased fibroblastic cell proliferation. Finally, Ora-PU, Lem-PU, and Tan-PU films inhibited the growth of *Escherichia coli* bacteria on their surface [1,2], and photodegradation of resveratrol occurred at levels of 2% (2 h), 8% (4 h) and 21% (8 h) in UVA, confirming their potential use as topical dressings.
Conclusions: The incorporation of citrus fruit peel extracts into PU films reduced the mechanical properties and showed no thermal event at body temperature besides decreasing the degree of swelling when compared to PU membrane; films were able to inhibit the growth of Gram-positive bacteria. Notably, the RSV was maintained atpeak levels of 98%-79% for 2-8 hours, when UVA radiation was applied. The results presented in this study reveal the potential application of citrus-Pu films for use in dressings protecting the wound and drug from UV radiation.

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CHITOSAN FILMS RETICULATED WITH LACTIC ACID AND FUNCTIONALIZED WITH NANO CHITOSAN, AS APROMISING BIOMATERIAL FOR BIODRESSINGS

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Keywords: Biomaterial; biofilm; Nano-Chitosan; bio-curative.

Introduction and objective: The investigation and standardization of techniques for developing safe and bioactive biomaterials hold significant importance. Existing literature supports the use of both chitosan and nanochitosan for this purpose [1]. Here the cytotoxicity of chitosan films crosslinked with lactic acid (with and without nano chitosan), was assessed in cell culture. Additionally, was established a standardized production technique, and the structure was examined by scanning electron microscopy. Furthermore, antimicrobial test was conducted to validate the aseptic method of preparation, and antibacterial tests evaluated its efficacy. Theobjective was to create a functional/safe biomaterial that can be effectively utilized as a biodressing.
Methodology: Films were made using a 300ml solution containing 1% lactic acid, 3g of chitosan powder [2], and 6,666% nano chitosan. Freshly made films were dehydrated (80ºC for 24 hours) before use. The films cytotoxicity (with and without nano chitosan) on cells was tested at three different time points (24h, 48h, 72h)by the Rezazurin microtiter assay - 570 nm test. Ultraviolet light was used to sterilize film samples, and these were tested on antimicrobial capacity, finally films were analyzed at the Centro Nacional de Pesquisa em Energia e Materiais (CNPEM) using Thermo Fisher Scientific INSPECT F50 Scanning Electron Microscope.
Results and discussion: The results, shows that the films freshly made when in contact with alcohol 70% and PBS
+ antibiotic solution where highly hygroscopic and exhibited some hydrogel aspects (gelatinous aspects) what led to size changes, so it must be dehydrated, in order to keep its mechanical properties. Data show that films at24h increased cell population by 22%, while at 48h it has 21% compared with the control group. Films without nano chitosan show an increase of 20% on cell population at 24h and the same at 48h in comparison with the control group. Furthermore, SEM images showed that the film is flat, with few changes on its relief that helps cell growth. Antimicrobial tests showed that 30 mins under ultraviolet light can make the film aseptic. Besides, antibacterial tests revealed that films without chitosan reduced *Escherichia Coli* population by 99%, while films with nano chitosan revealed a decrease in *Escherichia Coli* population of 53%.
Conclusions: Chitosan films cross linked with lactic acid, whether functionalized with nano chitosan or not exhibitpromising potential as a functional biodressing, once it is non-toxic for cells and has antimicrobial properties.

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ASSESSMENT OF PURE-PHASE β – TRICALCIUM PHOSPHATE (β – TCP) CYLINDRICAL CERAMIC IMPLANT OSSEOINTEGRATION USED IN CRITICAL SEGMENTAL BONE DEFECTS OF THE RADIUS OF RABBITS

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Keywords: biomaterials, β-TCP, ceramics, osteosynthesis.

Introduction and objective
Although grafts show excellent skeletal incorporation, host's morbidity and graft acquisition and quantity limit their use. Alternatively, synthetic materials play an important role in reconstructive orthopaedic surgery. To assess the occurrence of osseointegration using a customized β-TCP implant.

Methodology
Eighteen adult New Zealand rabbits were divided into 3 groups (n=6 per group). A defect was created in the mid-diaphysis of each left radius with a high-speed drill. In Group-A and Group-B, β-TCP and allogenous cortical bone grafts respectively were placed intro the radical defect, while the defect remained empty in Group-C. Pain, swelling, limb alignment, lameness, foreign body reactions, osseo integration and implant resorption were assessed through cãnical examination and qualitative radiographic, analysis immediately postoperatively and 30, 60, 90 and 120 days postoperatively. All animals were euthanized on day 120, and µCT and histological assessments for calcified and uncalcified specimens were performed.

Results and discussion
No clinical alterations were observed in any rabbit after 120 days. On radiographic and µCT images, as well as upon histologic assessment, complete bone healing was observed in all rabbits in Group-B. Non-unions were observed in all rabbits in Group-A and Group-C. Implant resorption was not observed in Group-A. This study showed that β-TCP did not elicit any osteoconductivity and osseointegration. The lack of ceramic bioactivity and resorption observed with β-TCP was probably due to low implant porosity and/or other implant shape features. However, expected biocompatibility was present. Our hypothesis was that by reducing porosity and gainingrigidity, we could have an alternative to maintain a good mechanical characteristic, without forcing and breaking the implant and at the same time, allowing blood vessels, cells and growth factors permeate the poresof the implant and form new bone throughout. But this feature was not enough to promote the conduction of bone cells nor the integration between them in our implant. Certainly, the porosity that we gained from the sintering process was too low to allow osseoconduction, which differed from the works of other authors.

Conclusions
The customized β-TCP implant did not show such osseoconductive and osseointegrative characteristics mentioned by other authors and they were not effective for the consolidation and integration between the hosts bones and biomaterials. Further studies must be carried out to provide more information to classify it as a proper material for use in the routine of orthopaedic reconstructive surgery in veterinary medicine.

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BIOACTIVE HYBRID SCAFFOLDS OF POLYCAPROLACTONE/KEFIRAN CONTAINING CaO NANOPARTICLES OBTAINED FROM EGGSHELLS FOR BONE TISSUE ENGINEERING

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Keywords: Bioactive scaffolds; polycaprolactone; kefiran; CaO nanoparticles

Introduction and objective: The worldwide increase in the elderly population has led to a rise in diseases like osteoporosis, resulting in bone fractures [1]. To address this clinical challenge, electrospun scaffolds using a combination of synthetic (such as polycaprolactone (PCL)) and natural polymers (such as kefiran) are attractive biomaterials [2]. These scaffolds can mimic the bone's extracellular matrix, promote cell adhesion and proliferation, possess similar mechanical properties as the tissue to be repaired, and are biodegradable [2]. Besides, to enhance bioactivity and integration with the host tissue, CaO nanoparticles can be incorporated to form hydroxyapatite. This study presents a novel approach to developing advanced functional nanofibrous PCL/kefiran/CaO scaffolds through a two-needle electrospinning method.
Methodology: Two polymer solutions were prepared separately, PCL (12.5% w/v in CF/DMSO 9:1) and kefiran (6 % w/v in deionized water). 5 wt% of CaO nanoparticles (± 5.2 nm) obtained from eggshells were added to the PCL solution. Each solution was placed separately in a 10 mL syringe to be electrospun at the same voltage (17 kV). Mats were characterized by scanning electron microscopy (SEM). Water absorption and biodegradability were performed by immersing the scaffolds in a phosphate-buffered saline (PBS) solution (pH 7.4). Bioactivity tests were performed to determine the hydroxyapatite (HA) formation by immersion in Simulated Body Fluid (SBF). The mechanical properties of the scaffolds were evaluated using the uniaxial tensile test.
Results and discussion: Hybrid scaffolds with high porosity and pore interconnectivity were obtained. The fibers had a random arrangement and a homogeneous surface free of defects. This suggests an efficient electrospinning process. The histograms of fiber diameter distribution indicated a bimodal-type distribution due to differences in the polymeric nature of the solutions. In addition, the presence of nanoparticles increased the fiber diameter, which can be explained by the increase in viscosity and conductivityof the PCL solution. The presence of kefiran and CaO nanoparticles notably increased the water absorption and affected the degradation of the scaffolds. The results suggested a hydrophilic material that can more easily lead to cell adhesion and growth. Young's modulus increased with the incorporation of CaO as reinforcement. The presence of kefiran and CaO in the scaffold (PCL/kefiran/CaO) induced rapid hydroxyapatite formation on the fiber surface compared to PCL/CaO scaffolds. This result indicated the synergistic effect of kefiran and CaO, suggesting that both provide a suitable chemical environment for HA forming, leading to improved bioactivity of the biomaterials.
Conclusions: The present study demonstrated that PCL/kefiran/CaO polymer-based hybrid scaffolds with interconnected fibrilar morphology could be fabricated by two-nozzle electrospinning. In addition, evaluation of their mechanical strength, water absorption capacity, biodegradation, and hydroxyapatite formation capacity on the surface confirms that these biomaterials possess the potential for bone tissue regeneration.

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LOADING OF CANNABIS SATIVA OIL EXTRACT ON HYDROGELS AND 3D PRINTED SCAFFOLDS FORWOUND HEALING

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Keywords: *Cannabis sativa*, hydrogels, nanoparticles, 3D printing

Introduction and objective: Nowadays, biomaterials with therapeutic molecules play an active role in wound healing and infection prevention. 3D printing approach has emerged to overcome several of the major deficiencies of tissue engineering (1). *Cannabis sativa* oil is known for its anti-inflammatory and analgesic effectsand antioxidant activity (2). The aim of this work was the development of hydrogels loaded with *cannabis sativa*oil using different approaches.

Methodology: We synthetized the collagen hydrogels and loaded with silver nanoparticles previously synthetized. Then the *Cannabis sativa* oil extract was added. For the characterization we performed transmissionand scanning electron microscopy, fourier transform infrared, silver absorption and release, rheological properties, enzymatic Degradation of Collagen Gels, antioxidant capacity, antimicrobial activity and biocompatibility. On the other hand, to develop 3D scaffolds, we first synthesized the gelatin and alginate bioink.We set the bioprinting parameters and after the impression we lyophilized the scaffolds and loaded them with *Cannabis sativa* oil extract. Finally, we performed the same characterization previously described for the collagenhydrogels.

Results and discussion: We were able to develop collagen hydrogels loaded with silver nanoparticles and *Cannabis sativa* oil extract. The presence of the silver nanoparticles gives interesting feature to the biomaterial such as improved mechanical properties, resistance to collagenase degradation and long-lasting antimicrobial effect. The antioxidant activity of *Cannabis sativa* oil successfully improved the biocompatibility and also enhances the antimicrobial activity against Gram positive and Gram negative bacteria during seven daysin liquid medium of the nanocomposite. On the other hand, we developed a bioink made with gelatin and alginate that was able to be printed using an extrusion 3D bioprinter. The 3D scaffolds obtained were able to belyophilized and when the elastic modes were assessed they show hydrogels properties. The swelling capacity ofthe 3D scaffolds was almost 800%. In this sense, the scaffolds were loaded with *Cannabis sativa* oil extract and the presence of the extract provided antimicrobial against Gram positive and Gram negative bacteria in both liquid and solid medium, and antioxidant activity to the 3D scaffolds.

Conclusions: Altogether, these results suggest that collagen hydrogels loaded with silver nanoparticles and *Cannabis sativa* oil extract are a promising alternative to common treatments of wound infections and wound healing. In addition, the new biocompatible material printed with 3D technology and with the addition of *Cannabis sativa* oil could become an attractive alternative to common treatments of soft-tissue infections andwound repair.

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INFLUENCE OF HUMAN TYPE I COLLAGEN ON THE PERFORMANCE OF ANIMAL-FREE BIOINKS FOR 3DBIOPRINTING AND CELL CULTURE: A COMPARATIVE STUDY

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Keywords: Collagen type I; Bioink; Animal-free; Bioprinting

Introduction and objective: Tissue engineering and biofabrication face the challenge of developing a bioink for 3D bioprinting free of animal inputs with good cell preference and adhesion. Studies show that the addition of type I collagen to bioink stimulates the structure of the printed fabric and promotes cell adhesion and viability[1]. The aim of this study is to evaluate different formulations of bioinks containing type I rat collagen (Corning®), type I human collagen (Qmatrix, Quantis®), at different concentrations.
Methodology: Primary human fibroblasts were cultured in animal-free medium (Quantis®) and then added to apatented hydrogel (Quantis®) followed by the addition of human and animal collagen to be studied, at a concentration of $2x10^6$ cells/mL. The solution was incubated for 4 days. Cellular/tissue morphology was assessed by light microscopy, while quantitative cell viability was assessed by Resazurin assay (0.01 mg/mL in solution) and cell viability by Live/Dead assay. Collagen quantification was performed with Picrossirius Red.
Results and discussion: The bioink containing QMatrix® showed low cytotoxicity, promoted cell migration and presented values up to two times higher in the positive feedback of collagen production, favouring adhesion and proliferation in the three-dimensional environment, compared to the other formulation containing animal collagen. It is estimated that Qmatrix® performed better due to its biocompatibility, providing cells with a human extracellular matrix microenvironment similar to the natural tissue environment. Even when dealing with the same protein, studies have already shown that small variations in the composition of amino acids can influence the physical properties of collagen, cell response and tissue remodelling. Currently, xenogeneic sources are widely used in tissue engineering, mainly due to the availability of options on the market, however,with advances in tissue engineering, it will be increasingly necessary to advance in the development of biocompatible and functional human inputs for medical applications.
Conclusions: In conclusion, it was observed that Qmatrix® (human collagen) showed significant regenerative characteristics in the 3D construct when compared to mouse collagen in bringing very promising perspectives for application in regenerative and esthetic medicine with great potential, for example, to act as a dermal and joint filler.

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BIOMIMETIC STICKER GELATIN-BASED HYDROGEL FUNCTIONALIZED BY PHENOLICCOMPOUNDS

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Keywords: Biomimetic; biomaterial; hydrogel; adhesive.

Introduction and objective: Biomimetics is a relatively new science that has great prospects in biomaterials field. This line of study aims to mimic living organisms to design sustainable new technologies. The focus of this researchis to develop biocompatible hydrogels with adhesion in wet tissues, inspired by a natural adhesion phenomenon observed in protein matrix excreted by marine blue mussel's foot *Mytilus edulis,* and investigate their potential astissue stickers for cutaneous injury treatment. Although the protein matrix complexity, it's known that catechol group of L-3,4-dihydroxyphenylalanine (L-DOPA) and high content of collagen show a relevant role in adhesion ability of mussel [1].

Methodology: In this work, four biomimetic adhesive hydrogels were synthesized in oxidant and basic reaction conditions, employing rutin and tannic acid polyphenols to substitute cathecol groups from DOPA and bovine gelatin to replace the collagen. Phenolic groups perform a crucial role in adhesion mechanisms and in chemical crosslinking through Michael's addition reaction with gelatin's amino groups [2]. The crosslinking reaction was investigated through the ninhydrin method and rheological analysis. Moreover, infrared spectroscopy and thermalanalysis investigated changes in gelatin structure. Mechanical tests were performed using porcine skin to evaluatehydrogel potential adhesion. Antifungal activity was studied against the pathogen *Trichophyton rubrum*.

Results and discussion: The results showed an effective synthesis of the bioinspired adhesive hydrogel and demonstrated the effect of crosslinking degree in its properties. Rheological data allowed for a quantitative assessment of the hydrogel's strength, correlating it with the degree of crosslinking and with the type of interaction between the polymeric chains within the network. Infrared spectroscopy analysis indicated modifications in secondary structure of the protein due to changes in the bands related to amide I e II. The crosslinking induces an increasing of glass transition temperature and affects the degradation profile of the materials. Furthermore, the hydrogels showed good adhesion in porcine skin after curing process at high relativehumidity and body temperature. Despite the low antifungal activity, the hydrogels exhibited a good dispersion matrix when incorporating a drug in the material.

Conclusions: This research successfully demonstrated the effective synthesis of adhesive hydrogels and highlightedthe importance of crosslinking degree in determining their properties. The physical-chemical, mechanical, and biological study provided valuable insights into the hydrogel's behaviour and performance. These biomimetic adhesive hydrogels have a promising prospect for applications in tissue engineering and drug delivery, offering an innovative and sustainable approach to biomedical materials.

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BACTERIAL CELLULOSE/ALGINATE SCAFFOLDS FUNCTIONALYZED WITH VITREOUSHUMOR AND EXTRACELLULAR VESICLES FOR WOUND HEALING

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Keywords: Aerogels; Vitreous Humor; Thunnus albacares; Extracellular Vesicles.

Introduction and objective :
Wound healing is a natural complex succession of events that aims to restore damaged tissue and its functions. By 2018 a US healthcare provider estimated that 8.2 million beneficiaries had wound-related demand [1]. This work aimed the development of new scaffolds for epithelial healing, biocompatible and biodegradable, using blends of bacterial cellulose (BC), oxidized bacterial cellulose (OBC) and alginate (AL). Additionally, as bioactives, were added vitreous humor (VH) from *Thunnus albacares* and complete conditioned media (CCM) from human dental pulp mesenchymal stem cells (MSCs).
Methodology: We formulated six different scaffold formulations: BC-AL-CCM; OBC-AL-CCM; BC-AL-HV-CCM; OBC-AL-HV-CCM; BC-AL-BSA-CCM; and OBC-AL-BSA-CCM. All scaffolds were evaluated by *in vitro*cytotoxicity against mouse fibroblasts (L-929), human keratinocytes (HaCat), and *in vitro* scratch test with L-929 cells. The scaffold morphology was evaluated using surface electron microscopy (SEM) and the images were analyzed using ImageJ software.
Results and discussion: We successfully formulated six aerogels with no cytotoxicity against both cell lines tested. All six presented cell viability between 70 - 115% after 48 h of contact with the cells, indicating no cell toxicity *in vitro*. The *in vitro* scratch showed the aerogels with HV and MCC changed the speed of the wound process and the pattern of cell proliferation/migration. The SEM images showed that the scaffolds were highly porous materials with an interconnected porous network throughout the materials. The surface porous size was modified by the presence of HV in the formulations.
Conclusions: So far, all formulations have shown results encouraging us to investigate their effects on epithelial healing further using *in vivo* models.

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NATIVE COLLAGEN EXTRACTION FROM TUNA SKIN BY ACID TREATMENT

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Keywords: Marine collagen; Type I collagen; Tuna skin; Fish By-product;

Introduction and objective:
Type I collagen makes up more than 90% of the collagen in the human body. It is present in the end product when tissue heals by repair, the scar tissue, skin, and connective tissues such as cartilages, bones, ligaments andtendons. It shows excellent properties such as biocompatibility, cell loading property and biodegradability, which make it an excellent choice for raw material of tissue engineering and regenerative medicine [1]. This study extracted collagen from tuna skin through ten consecutive steps, including acid treatment.
Methodology:
The collagen extraction was adapted from the method described by Pezeshk *et al*. (2022) [2]. The pre-treated skin received treatments in a jacketed reactor, cooled to 4 °C with continuous stirring. Starting with the basic treatment, where the skin was soaked into NaOH (0.1M), followed by the treatments with ethanol (20% v/v) and acetic acid (0.5 M). The supernatant was collected and vacuum filtered, precipitated with NaCl and dialyzed (MWCO = 14 kDa) until neutral pH was reached. Finally, the collagen solution was frozen in an ultra-freezer andlyophilized.
Results and discussion: The native collagen extraction from tuna skin was successfully performed with a product of very high purity as a result of the removal of non-collagenous proteins carried out with the basic treatment using NaOH and the removal of lipids by ethanol treatment, in addition to vacuum filtration and dialysis treatment, guaranteeing a purified product with a neutral pH. The collagen was extracted with a yield of 9.7% (dry weight basis).
Conclusions:
The results encourage us to investigate the use of this biopolymer, obtained as a product of this work, as a raw material in developing biomaterials for tissue engineering and regenerative medicine.

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THE IMPACT OF NON-DEPROTEINIZATION ON PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF NATURAL RUBBER LATEX FOR BIOMEDICAL APPLICATIONS

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Keywords: Latex, Hevea brasiliensis, Natural rubber, Proteins.

Introduction and objective: Latex is a milky liquid extracted from the Havea brasiliensis tree, which contains natural rubber, poly(isoprene), and other components such as proteins and phospholipids. These components are present in both natural rubber and latex serum. This study aimed to investigate the influence of the deproteinization process on the chemical and biological behavior of natural rubber latex. Natural Rubber (NR) extracted from the pure latex (LNCP) was obtained through centrifugation, and latex purification was performedusing solvent six times (LP6).

Methodology: For the analysis, membranes based on LNCP, NR, and LP6 were prepared. The LNCP group was used in its natural state, the NR group underwent prior centrifugation, and the LP6 group was purified six times using chloroform and precipitation with methanol. The structure was characterized using Fourier-transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), swelling test, Contact angle and Zeta potential.

Results and discussion: In the FTIR analysis, the absence of bands at 3400 cm-1 corresponding to OH/NH groupsand the presence of a band at 1545 cm-1 associated with proteins and phospholipids indicated that the LP6 sample was deproteinized and purified compared to the other groups [1]. DSC and TG analyses revealed that allthe analyzed groups did not undergo thermal degradation, meaning their chemical structure was unaffected. However, in the swelling assay, it was observed that the LP6 group experienced a mass loss over time, indicatinga visible change in the material's physical structure. Additionally, its swelling rate was relatively lower comparedto the other groups. The contact angle measurements demonstrated that the groups in question were hydrophobic, with the LP6 group exhibiting greater hydrophobicity compared to the other groups. This finding suggests that the exposure of isoprene units present in the polymer chain may result in an increased contact angle and reduced hydrophilicity [2]. The LP6 sample displayed a less negative surface charge compared to the other samples (NR and LNCP), reaching -13mV at pH 9.0. Consequently, the difference in zeta potential could beattributed to the deproteinization process employed in the LP6 sample, which rendered it less hydrophilic.

Conclusions: The results obtained provide evidence that latex purified with solvent 6 times (LP6) exhibited a more unstable material, with noticeable physical changes in its structure, increased swelling, and a reduced negative surface charge. The purification process has a significant influence on the physicochemical and biological behavior of the natural rubber latex. The proteins and lipids present in the LNCP group can positively influence its structure and enhance its performance in various medical and health applications.

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IMPLYING LOW TEMPERATURE PLASMA (LTP) TO DEVELOP SCAFFOLDS FOR TISSUE REGENERATION

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Introduction and objective: Around 40.5 % of the US population is expected to be affected by some form of cardiovascular diseases (CVD) by2030 [1}. Bypass surgery, using the autologous vein has been an effective treatments for CVD. Recently tissue engineering is gaining lot of usage for engineered vascular grafts to synthesize blood vessels Vascular grafts made of Dacron/ ePTFE does not work well for small diameter grafts (<6 mm) due to hyperplasia and thrombosis[2}. The present study is aimed to improve the endothelialization of intimal surface of graft by using low temperature plasma (LTP) to increase the cell attachment and proliferation.

Methodology: PTFE was treated with LTP. Air was used as the feed-gas and the pressure in the plasma chamber was kept at 800mTorr. Scaffolds were also modified with gelatin and collagen by dipping method. Human umbilical vein endothelial cells (HUVEC) were plated on the developed scaffolds and cell proliferation was determined by the MTT assay and by microscopy. mRNA expressions levels of different cell markers were investigated using quantitative real-time PCR (qPCR).

Results and discussion: XPS confirmed the introduction of oxygenated functionalities from LTP. HUVEC cells showed 80% seeding efficiency on the scaffold. Microscopic and MTT assays indicated increase in cell viability in LTP treated scaffoldsespecially when treated with gelatin or collagen compared to untreated scaffolds. Gene expression studies showsenhanced expression of cell adhesion marker Integrin- α 5 gene after LTP treatment.

Conclusions: LTP treated scaffolds exhibited better cell proliferation and viability compared to untreated scaffolds. Protein treatment of scaffold increased cell proliferation. Based on our initial results, more scaffolds alternatives will bedeveloped and investigated for cell growth and vascularization studies.

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STUDY OF CELL VIABILITY OF COMPOSITE POLYMER COATINGS OBTAINED BY EPD METHOD AND POROUS SCAFFOLDS OF PLA MANUFACTURED BY 3D PRINTING

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Keywords: biomaterials; tissue engineering; bioactive coatings; 3D printing

Introduction and objective: Biomaterials play an important role in tissue engineering because they provide new approaches to tissue repair or regeneration [1]. Composites made up of polymers, ceramics and bioactive phosphates are excellent candidates for these uses. Likewise, various methodologies capable of developing thesematerials have emerged, among which are fused deposition modeling (FDM-3D printing) and electrophoretic deposition (EPD) [2]. The aim of this work was to study the adhesion and viability of mesenchymal stem cells isolated from human periodontal ligament (phMSC) growing on composite coatings based on poly(ε- caprolactone-*block*-dimethylsiloxane) (PCL-*b*-PDMS) with tricalcium phosphate (TCP), and porous scaffolds of poly(lactic acid) (PLA).

Methodology: PLA scaffolds were fabricated by 3D printing, and PCL-*b*-PDMS/TCP coatings were obtained on stainless steel substrates through EPD technique. The bioactivity was evaluated by immersion in simulated body-fluid (SBF) for 7 and 28 days at 37 °C. Cell viability was studied in cultures using phMSC cells in 12-well plates with Dulbecco´s Modified Eagle Medium (DMEM), 10% of fetal bovine serum and Penicillin and Streptomycin antibiotics (5% CO_2 at 37 °C). After 3 days, the adhesion of the cells in scaffolds and coatings was verified by fluorescence microscopy. The phMSCs were isolated using the explant technique at a density of 100,000 cells/mL.Finally, cell viability assays were performed using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) technique.

Results and discussion: According to Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy (SEM- EDX), composite coatings are more compact and uniform at longer immersion times in SBF. Furthermore, EDX analysis revealed Ca/P ratio values between 1.4 and 1.50, close to the stoichiometric values of hydroxyapatite (HA) in human bone. The growth of HA can be attributed to the high diffusion of Ca ionic species relative to P, leading to an increased Ca release at the surface. On the other hand, Fluorescence Microscopy (Nikon TE 2000) analysis on porous scaffolds of PLA revealed 3T3-L1 cells that express the green fluorescent protein (GFP) growingon the porous scaffolds after 3 days of culture in DMEM supplemented with 10 % of FBS. Finally, cell viability results obtained through MTT assay revealed that both materials tested with phMSC cell exhibited a higher absorbance at 540 nm when compared to those without cell growth.

Conclusions: The in-vitro assessments evidenced the formation of crystalline structures corresponding to HA in the tested materials. On the other hand, cell viability tests showed the presence of viable phMSC cells in both analyzed materials. The viability of dental mesenchymal stem cells on PCL-*b*-PDMS/TCP composite materials andporous scaffolds of PLA obtained by 3D printing is demonstrated for the first time, making it of great interest forfuture applications of regenerative medicine in odontology.

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EXPANDED NANOFIBROUS POLYMERIC DRESSINGS CONTAINING TETRACYCLINE-LOADEDNANOPARTICLES FOR WOUND HEALING

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Keywords: electrospinning; polyvinyl alcohol; mesoporous silica nanoparticles; tetracycline

Introduction and objective:

The treatment of skin lesions often requires the use of temporary barriers to protect the wound from harmful agents and enhance the healing process. Electrospun polymeric nanofibers have emerged as a promising optionfor wound healing dressings due to their ability to mimic the extracellular environment of tissues [1]. In addition,these nanofibers can be easily modified to incorporate therapeutic and antibacterial agents, resulting in multifunctional materials. This study aims to investigate novel nanofibrous polymeric dressings with an expandedstructure, combined with mesoporous silica nanoparticles (MSN) as carriers of tetracycline, for potential application in skin tissue engineering.

Methodology:

MSN were synthesized by the sol-gel process [2]. The morphology, particle size and the specific surface area of MSN were characterized by SEM, TEM and BET analysis. Tetracycline was loaded into MSN via immersion of MSNin a concentrated drug solution, and the drug content was determined by TGA analysis and UV-Vis spectroscopy. The electrospinning parameters for PVA solutions were optimized, and different concentrations of MSN were incorporated. The resulting electrospun matrices were subjected to an expansion procedure in an ethanol solution of $NaBH_4$. The physicochemical, superficial, thermal and morphological properties of the nanocomposite materials were characterized.

Results and discussion:

The average size of MSN was determined to be 304 ± 35 nm, and TEM micrographs showed a highly ordered periodic porous microstructure. The nanoparticles show a Type IV BET adsorption isotherm, characteristic of mesoporous samples. TGA analysis demonstrated excellent thermal stability while the tetracycline-loaded MSNshowed a similar weight loss, albeit with a lower percentage. The loading efficiency of tetracycline into the MSNwas calculated to be approximately 9% (w/w). Tetracycline release from the MSN showed sustained release for approximately 15 h. Homogeneous fiber mats with randomly orientated and defect-free fibers were successfullyobtained for the hybrid scaffolds. To prevent the dissolution of PVA fibers in aqueous media, a thermal crosslinking process was applied. SEM micrographs revealed a homogeneous distribution of MSN within the fibrous mats, with a mean fiber diameter of 202 ± 60 and 185 ± 42 nm for PVA and PVA-MSN (3 wt.%), respectively. MSN incorporation resulted in a local broadening of the fiber due to its larger size. In addition, incorporation of MSN improved the thermal properties of the mats. The expanded materials exhibited an increase thickness due to the larger size and interconnectedness of the matrix pores.

Conclusions:

High loadings of tetracycline were achieved within the MSN, and electrospun PVA fibers with different MSN contents were successfully fabricated. The nanoparticles were uniformly embedded within the polymeric matrixalong the composite fibers. The crosslinked and expanded nanofibrous polymeric dressings exhibited improved mechanical properties, as well as increased pore size and interconnectivity. Future studies will focus on evaluating the antimicrobial properties of the expanded nanofibrous materials.

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BIOMECHANICAL CHARACTERIZATION OF FIBROUS POLYMERIC SCAFFOLDS FOR THE REGENERATION OFURETHRAL SEGMENTS

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Keywords: electrospinning; tissue engineering; urethra; mechanical properties

Introduction and objective:
Hypospadias and urethral strictures are among most common penile pathologies [1]. To address these conditions, the transplantation of autologous tissues, such as buccal mucosa and penile skin, has been commonly employed for urethral repair. However, this approach can be associated with potential complications, and there are limitations in the availability of donor tissue [2]. In this direction, tissue engineering offers a promising regenerative alternative to overcome these challenges.
The objective of this work is to investigate the mechanical properties of tubular scaffolds that combine syntheticand natural polymers, with the aim of exploring their potential for urethra regeneration.

Methodology:
The fibrous materials were fabricated by electrospinning and co-electrospinning using benign solvents. The polymer solutions employed include polycaprolactone (PCL) in acetic acid, poly(ethylene oxide) (PEO) in water/ethanol and PEO/hyaluronic acid (HA) aqueous solutions. Three different constructs were created: PCL scaffolds, PCL/PEO-HA scaffolds and PCL/PEO scaffolds infiltrated with HA. In the case of PCL/PEO scaffolds, thelow molecular weight PEO fibers were removed by water washing, followed by infiltration with a HA solution. SEM was employed to assess the fiber size and morphology, while the characteristic functional groups of the constructs were analyzed using FTIR. The mechanical properties of the scaffolds were evaluated using a continuous circulation biodynamic simulator. This allow for subjecting the urethral grafts to sinusoidal variations of internal pressure, mimicking the physiological bladder pressure ranges, and assessing their compliance and mechanical response *in vitro*.

Results and discussion:
SEM observations revealed that both PCL and and hybrid scaffolds exhibited homogeneous fibrous mats with randomly oriented and defect-free fibers. The mean fiber diameter for PCL mats, PEO-HA mats and hybrid PCL/PEO-HA scaffold were 1470 ± 720, 110 ± 20 and 830 ± 350 nm, respectively. The incorporation of PEO-AH nanofibers resulted in a reduction in the average fiber diameter for the hybrid scaffolds. The fiber size measurements of the PCL/PEO scaffold indicated a slight increase in the mean fiber size after washing of PEO fibers and HA infiltration. In the FTIR-ATR analysis, the signals associated to PEO disappeared after the washing step, and the characteristics bands of HA were detected after the infiltration process. The elasticity values of theconstructs were 3.36 ± 1.80, 0.61 ± 1.77 and 0.09 ± 0.17 Pa/m for PCL, PCL/PEO-HA and PCL/PEO infiltrated with HA, respectively.

Conclusions:
The fabrication of tubular structures through electrospinning is a promising technology to mimic the ECM and the biomechanical response of urethral tissue. By controlling the composition, structure and arrangement of fibers, it is possible to replicate the native tissue characteristics. The biomechanical characterization of the fibrous constructs demonstrated a performance comparable to synthetic scaffolds.

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BACTERIAL CELLULOSE/CALCIUM PHOSPHATES COMPOSITE CONTAINING CERIUMFOR BONE TISSUE ENGINEERING APPLICATIONS

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Keywords: Biocomposite; Bone repair; Hydroxyapatite; Polysaccharides.

Introduction and objective: Bacterial cellulose (BC) is a polysaccharide biosynthesized by acetic bacteria, extensively investigated due to its high mechanical strength, water uptake ability, non-cytotoxic and non-resorbable material. Calcium phosphates (CaP) are the most crucial bone mineral components and present biocompatibility, non-toxicity, bioactivity, and osteoconductivity. Several researchers have investigated the effects of incorporating metal ions into the CaP lattice, such as cerium (Ce) ions, that are recognized by accelerating bone neoformation [1]. This study aims to develop and assess the cell viability and *in vitro* degradation of bacterial cellulose/calcium phosphates (BC-CaP) and cerium-containing bacterial cellulose/calcium phosphate (BC-Ce: CaP) biocomposites for bone repair.

Methodology: BC-CaP and BC-Ce: CaP composites were prepared by successive incubation of the BC membranes into calcium and phosphate ions or calcium, cerium, and phosphate ions solutions, respectively. Three and six immersion cycles were investigated with 10 min of soaking in each solution. The content of cerium ranged from 5% to 15% (mol/mol). The samples were characterized by scanning electronic microscopy, energy dispersive spectroscopy, thermal analysis, X-ray diffraction, Raman, and infrared spectroscopy. MTT assessed the cell viability, and the degradation *in vitro* assay was performed by incubating the samples in phosphate saline buffer (pH 7,4 and t= 37°C).

Results and discussion: BC, BC-CaP, and BC-Ce: CaP with 5.0% (mol/mol) of Ce composites have presented cell viability and were characterized and investigated concerning the hydrolytic degradation profile. Thermogravimetric analyses revealed that the amount in the mineral phase was 73.62%, 74.91%, and 76.22%, respectively, for BC-CaP, BC-Ce: CaP (three cycles), and BC-Ce: CaP (six cycles), about the total weight. The analysis of X-ray diffraction, infrared, energy-dispersive X-ray, and Raman spectroscopy showed a formationof calcium phosphates, mainly monetite and hydroxyapatite phases, on cellulose nanofibers' surface. Infrared and Raman spectroscopies confirmed the presence of Ce-O vibrations in BC-Ce: CaP. The degradation assay demonstrated that bacterial cellulose could retain and absorb ions forming a layer of hydroxyapatite (HAp), predominant calcium phosphate in the materials studied, generating attractive morphologies such as urchin-likeand flower-like, which have been suggested in the literature for various applications related to bone tissue regeneration. The formation of a three-dimensional porous body for the BC-Ce-CaP (three cycles) composite wasobserved after 28 days of incubation in PBS with satisfactory pore diameters to assist neovascularization processes in tissues [2].

Conclusions: A novel BC-Ce: CaP composite was prepared, characterized, and assessed by cell viability and degradation in physiological conditions from 1 to 28 days. BC, BC-CaP, and BC-Ce: CaP with 5.0% (w/w) were not cytotoxic. SEM images corroborated the ability of BC to absorb PBS ions by biomineralization process, with an increase in weight after 21 days. Monetite, hydroxyapatite, and brushite were the main crystalline phases indexed. Urchin-like morphology was observed originating in a three-dimensional porous body, allowing neovascularization processes.

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IMPACTS OF DIFFERENT STRATEGIES FOR THE DECELLULARIZATION OF PORCINE AURICULAR CARTILAGE AS MODEL SCAFFOLDS FOR TISSUE ENGINEERING

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Keywords: Decellularization; tissue engineering; supercritical fluid; scaffolds.

Introduction and objective: Tissue engineering (TE) is a valuable approach to repair lesioned tissue and replace damaged organs, requiring suitable biocompatible scaffolds. These scaffolds should present adequate porosity, composition, and mechanical properties. The extracellular matrix (ECM) of animal tissues naturally exhibits these characteristics and has potential use in TE if efficiently decellularized. Supercritical fluids may better preserve mechanical properties compared to traditional surfactants like sodium dodecyl sulfate (SDS) in decellularization processes [1]. This study aimed to compare the impact of different decellularization strategies on the dimensions and mechanical properties of the ECM of porcine ear cartilage, which served as a tissue model.

Methodology: The skin was extracted from porcine ear and cylindrical samples of 6 mm in diameter were collected from equivalent regions of the cartilage tissue. Tissue decellularization was performed by exposure toSDS for 48 h, and to supercritical CO_2 ($scCO_2$) in the presence of either ethanol 70% (w/w), or butanol preceded or not by osmotic shock (OS) as a pretreatment to improve cell removal. The integrity of the ECM was analyzed by mechanical resistance tests at a maximum compressive stress of 80%. Changes in dimensions were determined with a pachymeter. Cell removal was verified by histological analysis with specific stains.

Results and discussion: Untreated cartilage samples collected from the same regions of auricular tissue regarding distance from head insertion exhibited statistically comparable mean properties in terms of thickness(1.7 ± 0.4 mm), mass (58.3 ± 7.8 mg), maximum compressive stress (MCS, 9.4 ± 0.9 MPa) and Young's module (YM, 16.7 ± 2.1 MPa) for different ears analyzed. When compared to data of the untreated biomaterial, the results of the different decellularization methods showed statistically significant variations (p-value<0.05) regarding several properties. An increase of 18% in thickness and an increase of 14% in YM were observed aftertreatment with $scCO_2$+ethanol 70%. Samples exposed to $scCO_2$+ethanol 70% preceded by osmotic shock increased 35% in thickness and showed a reduction of 24% in MCS. The treatment with $scCO_2$+butanol preceded by OS resulted in 17% decrease in MCS. All specimens suffered rupture below the compression limit of 80%, but $scCO_2$+butanol had the lowest impact. Cartilage diameters were not affected by the treatments.

Conclusions: SDS decellularization did not impact mechanical properties, but histological analysis showed insufficient cell removal for this method. Average diameters were affected for treatments involving ethanol 70%. The mechanical properties were not negatively impacted by $scCO_2$+ethanol 70% or butanol, but OS reduced MCS. Hence, $scCO_2$ associated to ethanol 70% or butanol are adequate to produce cartilage scaffolds applicable for TE or as model systems. The second process seems to be advantageous, preserving tissue thickness and YM and yielding higher compression resistance.

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EVALUATION OF GENOTOXICITY AND CYTOTOXICITY OF BIOSILICA AND SPONGIN DERIVED FROM MARINE SPONGES FOR 3D PRINTED SCAFFOLD PRODUCTION

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Keywords: biomaterials; biosilica; spongin; 3D printing

Introduction and objective: The utilization of natural materials as biomaterials have garnered significant attention due to their inherent qualities, including biocompatibility, biodegradability, and affordability [1]. Notably, marine-derived materials like biosilica (BS) and spongin (SPG) extracted from marine sponges have demonstrated promising outcomes in tissue regeneration [1]. Furthermore, 3D printing has emerged as an efficient technique for fabricating scaffolds using natural materials [2]. So, the aim of the present study was to evaluate the cytotoxicity and genotoxicity of 3D printed scaffolds of BS and BS/SPG.

Methodology: The marine sponges *Dragmacidon reticulatum* and *Aplysina fulva* were used for BS and SPG extraction, respectively. For scaffold fabrication, the Octopus™ - 3DBS bioprinter was employed and its morphology was presented through scanning electron microscopy (SEM). Cytotoxicity was indirectly analyzed byAlamarBlue® for 1, 3, and 7 days, using the extracts obtained from the scaffolds and adhesion test using SEM. Also, for the genotoxicity tests, comet assay and micronucleus methodologies were used. Two groups were formed: BS with biosilica scaffolds and a 4% alginate matrix and BS/SPG with biosilica and spongin scaffolds anda 4% alginate matrix, with concentration variations.

Results and discussion: In the SEM micrographs, it was possible to observe in the scaffolds of the BS group (BS 1, BS 2 and BS 3) the BS spicules are involved in the alginate matrix, and in the scaffolds of the BS/SPG group (BS/SPG 1, BS/SPG 2 and BS/SPG 3), in addition to the BS spicules, it is possible to notice that the matrix has a more fibrous aspect due to the mixture of SPG. In the AlamarBlue® analysis, none of the scaffold groups demonstrated cytotoxicity compared to the control (only with cells). Furthermore, in the SEM images, it was possible to observe the MC3T3-E1 cells adhered to the surface of the BS and BS/SPG scaffolds. Then, in the analysis of genotoxicity with CHO-K1 cells in the comet assay, no results were detected that could be related to a possible reaction of damage to the DNA of the cells in relation to the control. These results were confirmed through the micronucleus test with CHO-K1 cells. These results corroborate with studies involving BS from marinesponges and which demonstrated that it does not have any cytotoxic effect, in addition to having a good in vivoperformance [2].

Conclusions: The results of the present study demonstrated the great ability to use these two raw materials as natural biomaterials in bone tissue engineering. Thus, new in vivo studies will be needed to compare the performance of scaffolds from the BS and BS/SPG groups.

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RECENT TECHNOLOGICAL ADVANCES INTHE PROCESSING OFCONSTRUCTS TO OBTAIN MEAT GROWN IN LABORATORIES: A TECHNICAL, ECONOMIC AND ENVIRONMENTALFEASIBILITY STUDY(EVTEA)

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Keywords: Scaffold; electrospinning; cultivated meat.

Introduction and Objective: According to estimates from the World Resources Institute (WRI, 2023), the global population is expected to exceed 10 billion people by 2050, with a potential increase in demand for animal-based proteins by up to 70%. Currently, the agricultural sector in Mercosur is responsible for approximately 70% of greenhouse gas emissions, according to the Intergovernmental Panel on Climate Change (IPCC). Several studies have sought to optimize effective and cost-efficient methodologies for producing *scaffolds*, both for tissue engineering and for *in vitro* meat production through the three-dimensional cultivation of stem cells obtained from animal muscle tissue fragments. The objective of this work was to identify challenges and develop solutions for a sustainable substitute for animal protein consumption. The State of the Art of *scaffold* processing techniques for cultivating a protein ultrastructure (*in vitro* meat) was investigated to explore alternatives for food security through the use of three-dimensional culture matrices of food-based biomaterials. As the scope of this study, a critical analysis of technical, economic, and environmental viability (TEEV) was conducted, presenting future prospects regarding advances in this technology.

Methodology: Recent advances in *scaffold* development processes and their future prospects for large-scale production of *in vitro* meat were investigated in the literature. Two criteria were evaluated based on TEEV: scalability and economic feasibility of the process, which still depend on overcoming a series of technological obstacles. These obstacles include the isolation protocol for the most suitable cell types for efficient production processes, bioreactors equipped with advanced industrial-scale technology, consumer-safe products, and affordable costs.

Results and Discussion: The review presents relevant techniques for the proper production of *scaffolds* for cultured meat. It discusses the most promising techniques as well as the ones that still pose challenges, requiring further time and degree of study. Additionally, it identifies factors that new researchers can invest in for the advancement of the technique and future industrial-scale application.

Conclusion: Biomaterials are essential for tissue regeneration, and their application in innovative solutions is evident. *Scaffolds* for cellular growth in clean meat production have been extensively studied, and theirapplication is crucial for clean meat to become a new commodity. Technological advancements are increasing daily, and the process requires large-scale production means, which pose a challenge for the advancement of this biomaterial and effective clean meat production.

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IN VIVO BIOLOGICAL EFFECTS OF BIOSILICA AND MARINE COLLAGEN SCAFFOLDS ON THE PROCESS OFHEALING IN TIBIAL BONE DEFECT IN OSTEOPOROTIC RATS

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Keywords: Biosilica; Spongin-like collagen; marine sponges; osteoporosis

Introduction and objective:
Due to bioactive properties, the introduction of sponging-like collagen (SPG) into the biosilica (BS), both of them extracted from marine sponges, would present an enhanced biological material for improving osteoporotic fracture healing by increasing bone formation rate. Then the aim of the present work is to characterize the morphology of the BS/SPG scaffolds and to evaluate the orthotopic *in vivo* response of BS/SPG scaffolds into tibial defects of osteoporotic fractures in rats in 2 experimental periods, 15 and 30 days. The results presented that BS and BS/SPG scaffolds were able of improving bone healing. Furthermore, future research should focus on BS/SPG effects on long term periods in vivo studies.

Methodology: Scanning Electron Microscopy (SEM), the chemical bonds of the material by Fourier transform infrared spectroscopy (FTIR), (histology, histomorphometry and immunohistochemistry).

Results and discussion: SEM showed that scaffolds were porous, showing the spicules of BS and fibrous aspect of SPG. FTIR showed characteristics peak of BS and SPG. For the *in vivo* studies, after 30 days BS and BS/SPG showed a high amount of newly formed bone compared to the first experimental period, observed both in the periphery and in the central region of the defect. For histomorphometry, BS/SPG presented higher %BV/TV compared to other groups. Furthermore, after 15 days, BS presented higher volumes of collagen type I and after30 days, all groups presented higher volumes of collagen type III compared to 15 days experimental period. Additionally, after 30 days, BS/SPG presented higher immunostaining of osteoprotegerin compared to other groups at the same experimental period.

Conclusions: The results presented that BS and BS/SPG scaffolds were able of improving bone healing. Furthermore, future research should focus on BS/SPG effects on long term periods in vivo studies.

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USE OF HYDROGEL PATCHES OBTAINED BY ONE-POT PHOTOPOLYMERIZATION IN THETREATMENT OF A TORN SKIN WOUND IN AN EQUINE

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Keywords: dermatological patches; wound healing; hydrogels; biomaterial

Introduction and objective: Skin regeneration research faces the challenge of developing bioactive materials thatcan accelerate wound healing. Several factors, such as wounds in mobile areas, extensive tissue loss, and bacterial proliferation, can delay the healing process, particularly in equine injuries [1]. In such cases, secondary intention healing is necessary. Moist wound healing has proven to be an effective technique, and the development of hydrogel dressings has optimized this approach [2]. The objective of this study is to present an initial and local medical treatment for a torn skin wound on the hind limb of a horse using hydrogels and document its evolution.

Methodology: Patches were synthesized using photopolymerization with blue LEDs, comprising the following composition: MAA, METAC, and VCL at a concentration of 50% w/v, 2% w/w of SSOF-3, and 10% w/w of COL and HA. After synthesis, the materials were sterilized using a UV lamp. The patch was applied to an adult mare with a contaminated torn wound on the dorsal area of the left metatarsus. Antibiotic therapy and analgesia were administered during the first 5 days, and the bandage was replaced every 2 days. From day 10 onwards, the hydrogel patch became the sole treatment and was replaced weekly. The wound size was measured anddocumented photographically, and a sample was taken for histopathological study.

Results and discussion: The patches adhered to the wound bed with the assistance of a circumferential film layer,which remained in place until the next replacement. Pink granulation tissue was observed, filling the wound bed without excessive tissue growth. The measurements of the wound size demonstrated a reduction over time, withcomplete healing achieved at 116 days. No local or systemic clinical complications were observed throughout the therapy [1]. At the sampling on day 80, capillary hemorrhage and a velvety appearance of the wound were observed. Histopathological analysis of the sections stained with hematoxylin/eosin revealed mature granulationtissue with abundant collagen fibers, marked organization, and the presence of fibrocytes. Abundant capillaries were present in the connective tissue, along with hair follicles. No inflammatory reaction was observed. These results are promising, considering the mobile location of the injury, extensive tissue loss, and the possibility of bacterial contamination. The use of hydrogel patches containing MAA, METAC, VCL, COL, and HA accelerated thewound healing process and prevented the formation of excessive granulation tissue.

Conclusions: The application of this hydrogel patch promoted the healing process in a mare without altering the biomechanics of the hind limb, making it a practical and favorable therapeutic alternative for managing wounds in equines within their natural environment, while reducing clinical costs. Further investigation is warranted to explore appropriate physical support not only to maintain the hydrogel in the injured area but also to adapt it to each specific wound.

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WOUND TREATMENT: BIONANOCOMPOSITE FILMS TRANSFORMING FROM SOLID TO GEL STATE

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Keywords: COLAOB2023; biomaterials; wound infections; mechanical properties.

Introduction and objective: The skin, which is the most exposed tissue of the body, is susceptible to wounds of various magnitude and infections that require treatment. Therefore, the objective of this study was to obtain bionanocomposite films with antimicrobial and hemostatic properties that can transform into a gel state upon contact with wound exudate.

Methodology: The bionanocomposite films were obtained by the casting method using sodium alginate, montmorillonite and castor oil as components and loaded with different silver derivatives (AgNPs, Ag-Complex and $AgNO_3$). Rheological measurements were performed after wetting of the samples, using an Aton Paar rheometer. The kinetics of silver release from the films was performed in a Franz cell and was quantified by atomic absorption spectrophotometry. The *in vitro* biocompatibility of the films was determined using normal human dermal fibroblasts and red blood cells, and their antimicrobial activity against gram-positive and gram- negative bacteria was tested by the zone of inhibition method.

Results and discussion: Bionanocomposite polymeric films capable of transitioning from a solid formulation to a gel when exposed to skin exudate present in wounds have been successfully synthesized. This is demonstrated by rheological measurements of the wetted films, where the storage modulus (G') remainsabove the loss modulus (G''), indicating the formation of a gel [1]. Silver was released from the films following prolonged release kinetics and through different mechanisms depending on the silver derivative loaded in the film. Furthermore, a good biocompatibility of the bionanocomposite films was demonstrated *in vitro* with NHDF cells, with viability percentages higher than 80% in all cases. As for red blood cells, hemolysis was observed to be less than 2% in all cases. As regards antimicrobial activity, good activity was found against the three types of bacteria tested: *S. aureus* (Gram positive), *P. aeruginosa* and *E. coli* (Gram negative).

Conclusions: Biocompatible bionanocomposite films were obtained that are capable of prolonged silver releaseand have the particularity of transforming into a gel upon contact with wound exudate. These films offer the combined advantages of solid formulations, such as storage stability and dosage uniformity, with the benefits of gels, such as the relief of burning or itching sensation, as well as the ability to maintain skin hydration and maintain therapeutic antimicrobial activities for prolonged times in skin wounds.

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BIODEGRADABLE SCAFFOLDS WITH *Jatropha mollissima (Pohl) Baill* EXTRACT. FORUSE AS DRESSINGS

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Keywords: Biomaterials; Biodegradable scaffolds; dressings; epithelial regeneration.

Introduction and objective: Obtaining innovative biomaterials from biomass is extremely important for the development of research in the Northeast region and in Brazil as a whole. This approach promotes sustainability, regional development, valuing biodiversity, and creating new industrial and technological applications, in addition to contributing to the health and well-being of the population. Investing in this area is a way to boost the country's science, economy, and progress. Thus, this work aimed to develop biodegradable scaffolds of chitosan, collagen, and ethanolic extract of Jatropha mollissima for use as dressings.
Methodology: The lyophilization method obtained Chitosan scaffolds with collagen in different concentrations (5, 10, and 30%) of Jatropha mollissima´s ethanolic extract. They were then submitted to Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), porosity, biodegradation, and biocompatibility tests.
Results and discussion: In the Fourier transform infrared spectroscopy test, characteristic functional groups of the raw materials used were identified and the scaffold spectra show the mixture of precursor materials in all developed compositions since the characteristic bands of chitosan, collagen, and ethanolic extract of Jatropha mollissima have been observed [1]. Through morphological analysis, it was verified the presence of interconnected pores, with irregular shapes and sizes in all compositions studied. Mean porosity values did not show a statistical difference ($p = 0.425$), on the other hand, mean pore size was significantly affected by the incorporation of Jatropha mollissima ethanolic extract ($p < 0.005$). The average pore size is within the range reported as ideal for fibroblast adhesion and proliferation. Porosity plays an essential role in cell migration and proliferation, as it influences the exchange of gases and nutrients. The enzymatic biodegradation test showed amaximum mass loss of approximately 40% in 28 days. In the biocompatible assay, all studied samples proved tobe as biomaterial.
Conclusions: According to the literature, the ideal pores for the cultivation of soft tissues should be above 100 µm. Morfological, textural, and biocompatible analysis showed that scaffolds used in this research could be recycled, as would dressing.

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BIOPOLYMERS AND JATROPHA MOLISSIMA EXTRACT SCAFFOLD FOR DIFFICULT WOUND HEALING TREATMENTS

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Keywords: Tissue Engineering; Biopolymers; Dressings; Epithelial regeneration.

Introduction and objective: This study highlights the potential use of biopolymer-based scaffolds (chitosan and collagen) combined with *Jatropha mollissima* extract for treating challenging wounds. These scaffolds improve the mechanical properties of biopolymers with the bioactive characteristics of the extract, creating a favorable environment for tissue regeneration and wound healing. However, further research is needed to evaluate the effectiveness of these scaffolds on different wound types and their practical application on a large scale. Therefore, this research aims to develop chitosan and collagen-based scaffolds infused with ethanolic extract of*Jatropha mollissima* for use as wound dressings.
Methodology: Chitosan and collagen scaffolds were obtained using the lyophilization method. Various concentrations (5%, 10%, and 30% w/w) of *Jatropha mollissima* extract were incorporated into the scaffolds. The raw materials underwent organoleptic evaluation, Fourier transforms infrared spectroscopy (FTIR), antimicrobial activity, cytotoxicity, and pH analysis. Additionally, column chromatography and thin-layer chromatography were performed on the extract. The resulting scaffolds were subjected to FTIR analysis, swelling and degradation tests, as well as biocompatibility assessments.
Results and discussion: FTIR analysis revealed spectra corresponding to the raw extract and its ethanolic extract, with identifiable bands representing flavonoids, saponins, amino acids, and phenols. This indicates a high concentration of these substances in the extract. The FTIR analysis of chitosan and collagen showed characteristic peaks that aligned with existing literature [1]. Column chromatography of the organic fractions resulted in 5 isolated substances and 14 mixtures, all weighing less than 20 mg. Thin-layer chromatography demonstrated improved substance separation when altering the mobile phase's polarity. The antimicrobial activity test using the disk diffusion method indicated that chitosan exhibited activity against *Staphylococcus aureus* and *Escherichia coli*, while the extract demonstrated antimicrobial activity specifically against *Staphylococcus aureus*. The cytotoxicity assay demonstrated the biocompatibility of the materials under investigation. The extract was found to be acidic, and when combined with the biological properties of *Jatropha mollissima*, it enhances the potential of the scaffolds for the treatment of difficult-to-heal wounds.
Conclusions: The achieved properties of the chitosan and collagen scaffolds, in combination with *Jatropha mollissima*, demonstrated their potential application in tissue engineering for the treatment of complex wounds. The results of this research can contribute to the establishment of effective health promotion strategies, aimedat the population, by reducing the costs associated with the public health system and minimizing the risk of amputations in patients with chronic wounds.

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CHITOSAN/COLLAGEN HYDROGEL FOR WOUND TREATMENT

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Keywords: biomaterials; hydrogel; chitosan.

Introduction and objective: The treatment of chronic or difficult-to-heal wounds plays a crucial role in improving patients' quality of life. Hydrogel development has emerged as a prominent approach due to its capacity to effectively promote wound healing. By utilizing biocompatible and biodegradable materials capable of controlled release of growth factors, hydrogels can accelerate the healing process, reduce infection rates, and enhance tissue regeneration. This study aims to develop a chitosan and collagen-based hydrogel for woundtreatment.

Methodology: Chitosan and collagen hydrogels at concentrations of 1%, 3%, and 5% (based on the mass of chitosan) were prepared through mechanical stirring. Collagen was initially solubilized in acetic acid using an ultrasonic processor. After obtaining the hydrogels, they were subjected to centrifugation and characterized using Fourier Transform Infrared Spectroscopy (FTIR), viscosity measurements, and injectability assessments.

Results and discussion: The FTIR analysis revealed that the hydrogel with a 5% collagen concentration exhibited the highest intensity in collagen-specific peaks, such as those associated with proline and hydroxyproline (1450 cm^{-1}), as well as the band between 1300 and 1200 cm^{-1} attributed to the tripeptide (Gly- Pro-Hyp). These results are consistent with existing literature [1]. The injectability test demonstrated stable compressive loads below the recommended limit of 30N, suggesting the potential for use in manual injectable systems. Viscosity analysis, a critical property ensuring better adhesion of the hydrogel to the treated area, indicated predominantly viscous and pseudoplastic behavior, supporting the injectability test findings.

Conclusions: Varying concentrations of chitosan and collagen, which are derived from an animal-based fibrous protein, influenced the chemical and mechanical properties of the hydrogels evaluated in this study. By employing the proposed methodology, chitosan/collagen hydrogels were successfully obtained and characterized. These preliminary results reinforce the potential therapeutic efficacy of these hydrogels for the treatment of chronic or difficult-to-heal wounds.

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CO-ELECTROSPINNING OF BIOGLASS-LOADED PCL NANOFIBERS AND AMOXICILLIN-LOADED EUDRAGIT EPO NANOFIBERS AS COATING FOR METALLIC IMPLANTS

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Keywords: bone regeneration; co-electrospinning; nanofibrous coating; antibiotic

Introduction and objective: The use of metallic bone implants requires surface modification to improve the material properties, e.g., by using composite polymeric coatings. Electrospinning technique is highly recommended because the nanofiber structure mimics the extracellular matrix. Poly(ε-caprolactone) (PCL) is biocompatible and biodegradable, and bioglass (BG) can be included in the fibers providing osteogenesis and angiogenesis features. Moreover, pH-responsive fibers loaded with amoxicillin (AMX) were electrospun using Eudragit-EPO (EEPO), that dissolves at pH<5. If infection occurs, this condition takes place [1] and the fibers are expected to release antibiotics. The aim of this work was to fabricate nanofibrous implant coatings using co- electrospinning.
Methodology: 5wt% BG was homogeneously dispersed in a solution of PCL (20wt%/v) prepared in glacial aceticacid. A solution of EEPO (25wt%/v) was prepared in a DMF/ethanol mixture. 1wt% AMX was dissolved in the EEPO-solution. A homemade co-electrospinning setup was used. Rotating 316LVM wire substrates (1mm diameter) were used as collectors. PCL+BG and EEPO+AMX solutions were loaded into 10mL syringes and electrospun with certain flow rates, needle-target distance, and voltage parameters. Basic hydrolysis followed by exposure to UV-radiation at 254nm were carried out to hydrophilize and disinfect the surface. The physicochemical and antibiotic behavior of the coating was evaluated.
Results and discussion: Tubular nanofibrous coatings were successfully co-electrospun with the proposed deviceset-up. The coated implants could be easily cut with regular pliers after processing and the nanofibrous coating exhibited excellent coverage. To test the behavior of Eudragit-EPO at acidic pH, wires coated only with EEPO+AMX nanofibers were immersed in PBS pH 5.5, and, in less than one minute, dissolution of the coating wasobserved, leaving the metal exposed. The fiber diameter analysis of the SEM images showed a wide distribution,with the contribution of large PCL+BG fibers and smaller EEPO+AMX fibers. Furthermore, a partial preferential orientation of certain fibers could be noted, because of the small diameter of the rotational collector and the rotation speed. In addition, SEM images showed that after one-day immersion in PBS at pH 5.5, the PCL fibrous structure of the co-electrospinning coating maintained its integrity. FTIR-ATR analysis showed the main bands related to the polymers, as major components, that remained practically unchanged after the surface treatments. The antibacterial behavior of the implant was studied against *E. coli* and *S. aureus* at pH 5 and 7, in LB medium. The antimicrobial activity exhibited variations according to pH, type of bacteria (gram-positive/gram-negative) and contact time.
Conclusions: Biomimetic coatings were produced with a sample geometry that is highly beneficial for subsequent in vivo tests in rat model as hybrid orthopedic implants. The use of co-electrospinning improved the characteristics of the implant, allowing the release of antibiotics that mitigate or eliminate infections that couldtake place during surgical procedures.

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DEVELOPMENT AND *IN VITRO* EVALUATION OF BIOMATERIALS PRODUCED BY ROTARY JET SPINNING BASED ON PCL AND PHB WITH ADDITION OF AMAZONIAN VEGETABLE OILS

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Keywords: Tissue engineering; scaffold; fixed vegetable oils; cell viability.

Introduction and objective: Scaffolds help in the regeneration of bone tissue with anomalies or fractures. Among different scaffold production techniques, we can mention Rotary Jet Spinning, using different polymeric materials such as poly(ε-caprolactone) (PCL) and polyhydroxybutyrate (PHB), known for their biodegradability and biocompatibility. The use of fixed vegetable oils in scaffolds, rich in bioactive compounds, can add several benefits: antioxidant, anti-inflammatory and healing properties. Therefore, the objective of this study was to obtain PCL and PCL/PHB membranes with or without pracaxi oils, açaí oils or their mixtures, in addition to evaluating cell viability, for potential use in bone tissue engineering.
Methodology: The scaffolds were produced by the Rotary Jet Spinning technique, using PCL and PHB polymers. Açaí oil (AO) and Jupati oil (JO) were added to the material using the immersion technique. The morphological characterization of the material was performed using the scanning electron microscopy (SEM) technique. The cytotoxicity of the developed polymeric materials was evaluated in vitro, in murine fibroblasts.
Results and discussion: The morphological characterization of the material (SEM) showed a variation in fiber diameters. Fibers with only PCL had the largest diameters, ranging from 14,311 to 52,100 µm, with many pores. However, in materials with AO and JO, there was a reduction in fiber diameters. For PCL fibers with the addition of PHB, there was a decrease in diameters (ranging from 6.053 to 26.776 µm), in addition to high porosity. All fibers to which jupati, açaí oils or their blends were added showed pore obstruction, due to the deposition of oils on the surface. This feature can interfere with cell adhesion and diffusion of substances at the membrane/tissue interface [1]. The scaffolds produced from PCL only showed 82% cell viability. When combined PCL+PHB, the viability increased to 98%, and in materials containing PCL+JO, the result was 80%. For PCL+PHB+JO materials,
>98% response was obtained. On the other hand, there was a reduction in cell viability in the material containing PCL+AO (about 58%). However, when AO was combined with the PCL+PHB material, there was an improvement in viability (> 98%).
Conclusions: PCL/PHB scaffolds showed the smallest fiber diameters and best cell viability results when combined with oils. In particular those combined with JO exhibited better cell viability in vitro.

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SYNTHESIS OF A NEW ANTIBACTERIAL BIOMATERIAL CONTAINING HYDROXYAPATITE / CLORHEXIDINE FOR FUTURE APPLICATIONS IN BONE TISSUE ENGINEERING

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Keywords: Bone repair; Hydroxyapatite; Gallium; Antiseptic; Antibacterial properties.

Introduction and objective : Biomaterials, including calcium phosphate-based materials, have been extensively studied for their applications in the restoration of bone and dental tissue. Hydroxyapatite (HA) is a widely used bioceramic as a result of its excellent osteoinductive, osteoconductive, and osteogenic properties. Modifications such as dopant substituents and surface functionalization can enhance their antibacterial effects and drug interactions. Gallium (Ga^{3+}), a bioactive cation, can enhance the physical, chemical,and biological effects of biomaterials. This study focuses on the synthesis and characterization of gallium- containing hydroxyapatite (Ga-HA) and a Ga-HA/chlorhexidine (CLX) biomaterial. The antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*, as well as the cytotoxic effects, were evaluated using differentconcentrations of CLX adsorbed on the surface of Ga-HA.

Methodology: In this study, two synthesis methodologies were employed. First, pure HA and Ga-HA were synthesized using the suspension precipitation method. For the synthesis of HA, Ca (OH) 2 and $(NH_4)_2HPO_4$ were dissolved in deionized water (3h at 50 °C, pH = 10-11). The synthesis of Ga-HA involved the addition of $Ga(NO_3)_3$ to the HA precursor solution. In the second step, chlorhexidine (CLX) was incorporated into the HA and Ga-HA surfaces via adsorption. The materials were characterized by X-DR, XPS, FT-IR, and FE-SEM. Antibacterial activity (direct contact technique) and cytotoxicity (MTT colorimetric assay) were carried out to evaluate the performance of the materials.

Results and discussion: XRD patterns showed that the materials showed good crystallinity andexhibited planes characteristic of the hydroxyapatite phase. The lattice parameters of Ga-HA were slightly increased compared to pure HA, indicating the possible incorporation of Ga^{3+} ions into the structure. XPS analysisof Ga-HA confirmed the presence of characteristic peaks corresponding to hydroxyapatite elements. The bindingenergy identified in the XPS around 24 eV reveals that the peak refers to the excited electrons of the 3d orbital of Ga [1]. The Ca/P ratio and the (Ca+Ga)/P ratio were calculated using data from the XPS analysis. The calculatedvalues were 1.50 and 1.72, respectively. FT-IR analysis of the Ga-HA/CLX material revealed characteristichydroxyapatite bands and additional CLX-related bands [2]. The FE-SEM images showed clusters of non-uniformparticles with an irregular surface. When evaluating the antibacterial and cytotoxic effects of functionalized materials at the following concentrations of CLX: 0.20% v/v, 0.80% v/v and 1.20% v/v, it was observed that only HA / CLX- 0.20 did not show 100% inhibition against *Staphylococcus aureus* and *Escherichia coli*. The materials showed cell viability between 35±5% to 81±3%. Ga-HA / CLX-0.20 material was classified as noncytotoxic and theGa-HA/CLX-1.20 was classified as highly cytotoxic.

Conclusions: Incorporation of CLX on the surface of HA allows the development of a new biomaterial that combines the antimicrobial properties of CLX with the bioceramic applications of HA. Characterization techniques confirmed the successful synthesis of hydroxyapatite and the efficient incorporation of CLX into powder surfaces. Interestingly, even at low CLX concentration (0.20% v/v), the antimicrobial action was 100% effective, and Ga-HA/CLX-0.20was classified as noncytotoxic after 24 hours of cell incubation.

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DEVELOPMENT OF CHITOSAN CONTAINING VEGETABLE OIL SCAFFOLDS BY 3D PRINTING AIMING CHRONIC EPITHELIAL WOUND REPAIR

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Keywords: chitosan; vegetable oil; 3D printing; epithelial wound repair.

Introduction and objective: Wound healing can be disrupted, slowing down the healing process and thus resulting in chronic wounds. In the context of skin repair, chitosan is a promising natural polymer, with antimicrobial and antioxidant properties. Such materials can form hydrogels, being suitable for 3D printing process. Vegetable oils are in general composed of essential fatty acids, vitamins, and minerals that can contribute to wound healing. In this work *scaffolds* based on chitosan incorporated with different concentrations of vegetable oil were produced by 3D printing, aiming at the healing of chronic epithelial wounds.

Methodology Hydrogels were produced by varying the vegetable oil percentage in the chitosan matrix. They were used as ink for 3D printing aiming the production of scaffolds. The mechanical properties of these structures were studied through tensile behaviour of wet and dry scaffolds. Indirect cytotoxicity, adhesion, and proliferation assays up to 96hs were carried out using balb/c 3T3 cells to explore the biological properties of the material.
Results and discussion The printed scaffolds presented good shape fidelity when compared to the CAD models and displayed better results with the vegetable oil incorporation in the chitosan hydrogel. The tensile experiments showed that the dried scaffolds have higher mechanical properties and behave as soft materials in wet conditions. The *in vitro* toxicity assay results showed that the degradation products of the scaffolds were non-toxic, and increased cell viability in a dose/dependent manner. Vegetable oil increased cell adhesion of the chitosan scaffold, with better results at lower oil concentrations. The proliferation assay showed better results for chitonsan containing vegetable oil in early stages. However, for 96hs, pure chitosan have better cell proliferation.
Conclusions: Scaffolds of chitosan containing vegetable oil have potential applications as wound dressings, acting in physical and microbiological protection and cell proliferation of the tissue. Other biological assays are indicated to confirm the possibility of applying these biomaterials in chronic wounds.

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CHITOSAN HYDROGEL CONTAINING BIOACTIVE GLASS AIMING AT 3D PRINTING FOR PERIPHERALNERVE REGENERATION

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Keywords: 3D printing; chitosan; bioactive glass; peripheral nerve regeneration.

Introduction and objective: Peripheral nerve lesions are a challenge in the context of regeneration. Tissue engineering strategy can increase repair success using biomaterials and cells. Chitosan is a biocompatible natural polymer, with good cost-effectiveness and rheological properties that has been shown to be suitable for nerve regeneration. Bioactive glasses exhibited the ability to increase the production of neurotrophicsubstances in *in vivo* experiments that could assist nerve repair. 3D printing presents the possibility of building complex and patient-specific structures. Thus, the development of chitosan hydrogels with different concentrations of bioactive glasses for 3D printing is proposed for future application in peripheral nerveregeneration.

Methodology: Chitosan hydrogel was produced with different concentrations of bioactive glass (0, 0.5%, 1%, 2%, and 5%). The rheological characterization of the hydrogels was carried out by shear sweep and creep- recovery experiments. Printability and shape fidelity were evaluated after the 3D printing process through the comparison of the theoretical CAD model and printed structure. Indirect cytotoxicity assay using PC12 cells wasperformed to evaluate the biological properties of the scaffolds.

Results and discussion: The presence of glass affects the rheological properties of chitosan hydrogel. The shear sweep results showed that the viscosity of the chitosan containing bioactive glass decreases at the lowest shearrate compared to pure chitosan hydrogel. However, the viscosity of hydrogels containing higher concentrations of glass (2% and 5%) increases when compared to the other composites. Such behaviour is a consequence of two events: the presence of the bioactive glass particles and the ions release, caused by glass dissolution in the material, which can interact with the polymeric chains and disrupt the formation of intermolecular interactions. Furthermore, the incorporation of the ceramic particles increased the recovery percentage of the material. This raise is caused by the interactions between the bioactive glass surface and chitosan chains, increasing the hydrogel's stability. These rheological characteristics improved shape fidelity of the material and possible use as ink for 3D printing. Biologically, the extracts of the materials had a non-toxic effect on the PC12 cells.

Conclusions: The presence of glass affects the rheological properties of chitosan hydrogel to improve its use as 3D printing ink. The biological experiments showed that the materials do not have cytotoxic effects and could be used in tissue engineering. Therefore, chitosan hydrogel with bioactive glass particles are promising materials for application in peripheral nerve regeneration.

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MESOPOROUS SILICA ELECTROSPUN MEMBRANESFOR BONE TISSUE ENGINEERING APPLICATIONS

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Keywords: mesoporous silica nanofibers; electrospinning; sol-gel; bone tissue engineering.

Introduction and objective: Silica-based materials exhibit biocompatibility and biodegradability, making them suitable for long-term support in tissue regeneration. In particular, mesoporous silica can also be considered bioactive due to its large surface area that exposes a high density of reactive silanol functional groups [1].These groups facilitate a rapid release of soluble silicon in the form of silicic acid as a result of ion exchange in physiological fluid. Moreover, this behavior enables the adsorption of glycoproteins, which in turn can enhance cell adhesion, proliferation, osteoblast expression, and mineralization [2]. Therefore, the aim of this study is to obtain a mesoporous nanostructured silica membrane with high specific surface area and fibrous morphology for potential use in bone tissue engineering applications.

Methodology: In this study the following procedure was followed: (1) Preparation of a homogeneous, spinnable sol-gel precursor, (2) Electrospinning under appropriate conditions (humidity (HR), temperature, distance to collector, voltage, flow rate), (3) Heat treatment: membranes were dried at 60ºC for 1 h and calcined at 550ºC (2ºC/min) for 3 hs. TEOS was employed as silica precursor, Pluronic P123 as the structure-directing agent to generate ordered mesoporosity, ethanol as the common solvent, and poly(vinylpyrrolidone) (PVP) to increase the viscosity. H_2O/HCl was added dropwise to initiate the 30 min-prehydrolysis at room temperature. Then, the hydrolysis/condensation reaction continued for 3 hs at 80ºC under reflux. The H_2O:TEOS:HCl molar ratio was fixed at 2:1:0.01, which favors the formation of fibers.

Results and discussion: The preparation of mesoporous silica nanofibers by the coupling of the sol-gel/EISA (evaporation-induced self-assembly method) synthesis with the electrospinning process is rare in literature, since it is a challenging work which requires a sophisticated control over a wide number of parameters. Therefore, several conditions were tested in this work. The best results were displayed by a solution with the following molar ratio: EtOH : TEOS : P123 : PVP (15 : 1 : 0.012 : 2.75 x 10^{-5}), processed at 50 % HR, flow rate of1 mL/h, applied voltage of 14 kV and distance to collector of 15 cm. The obtained membrane presented a large BET surface area of 260 m^2/g, displaying a hybrid isotherm between type I and type II and a H4 hysteresis loop, typical of slit-shaped pores. The sample showed a broad pore size distribution with meso (V_{MP} of 0.1 cm^3/g) andmicro-pores ($V_{\mu p}$ of 0.05 cm^3/g). SEM images showed randomly arranged fibers in a nonwoven network, while TEM images exhibited fibers with disordered, worm-like mesoporous structure.

In the absence of P123 membranes were less brittle. TEM images revealed smoother fibers with no observable mesoporous structure. These membranes showed a type I isotherm, with a higher surface area (400 m^2/g) due to microporosity ($V_{\mu p}$ of 0.13 cm^3/g).

Conclusions: Self-standing fibrous silica membranes with high surface area were successfully obtained through the combination of electrospinning and sol-gel/EISA methods. Despite exhibiting a large mesopore volume, the interference of the electric field and the rapid solvent evaporation during electrospinning may have a negative effect on the formation of an ordered mesoporous structure. Therefore, further work will be performed to improve the structural integrity and achieve a more ordered mesoporous configuration.

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SECOND-DEGREE BURNS TREATMENT: PRECLINICAL APPROACHES USING DEPROTEINIZED LATEX NATURAL RUBBER CONTAINING *ALOE VERA* EXTRACT

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Keywords: Herbal medicine; wound dressing; drug release; natural latex rubber; Aloe vera; skin healing

Introduction and objective: The latex rubber obtained from Hevea brasiliensis is low cost and has high potential in pharmacological approaches. Despite the allergenic proteins, when chemically removed, the final product is flexible, can be molded into membranes and incorporate bioactive molecules [1]. Aloe vera gel (AV) is a natural bioactive compound that has strong anti-inflammatory and microbicidal effects [2]. After deproteinization, naturallatex rubber (NLR) with an AV creates a candidate to treat wound healing. However, the objective of this work is to evaluate the biocompatibility of mesenchymal stem cells developed in a dressing and its application in second-degree burns on the skin of the back of rats. **Methodology:** First, the latex was purified to remove allergenic protein residues and solubilized to 10%. The VA were added to the latex solution at concentrations of 0%, 25, 35 and 50% AV and the membranes were producedby casting. AV release assay was performed by UV-VIS, biocompatibility was assessed by MTT assay and live/dead fluorescent vital dye by Confocal Microscopy. The in vivo assays were performed on the skin of rats submitted to burns at 72°C for 10 seconds. Soon after marking the injured area, the dressing was added for 14 days. Afterwards,the animals were euthanized, and the skin was processed and analyzed by histology for morphometric and morphological studies. **Results and discussion:** The formulations showed biphasic release patterns with an initial burst of AV release followed by sustained release after 180h. The data obtained indicated rapid initial AV release rate with values of 23.4, 37.4 and 73.6 $mg.h^{-1}$ for reported AV concentrations. In the in vitro assays, there was a reduction in thenumber of cells over time only in the latex group containing 50% AV. There was a considerable effect on cell metabolism in both the MTT assay and the Confocal assay. In the histological analysis of the control groups, weidentified that the presence of latex in skin regeneration minimized the inflammatory process, evidencing its benefit in the treatment of burns. When associated with AV in concentrations, it showed a regenerating potentialand an angiogenic effect, with therapeutic potential for the treatment of grade I or II burns. Due to the toxicity found at 50% in vitro, in vivo tests were performed with latex at 35% AV. After 14 days, there was completerecovery of the epithelial tissue of the cutaneous lining of all animals with a dressing containing 35% AV. However,in animals containing only latex, recovery occurred with partial and not total neoepithelium in the injured areas. **Conclusions:** The results showed that the presence of latex in skin regeneration minimized the inflammatoryprocess, evidencing its benefit in the treatment of burns and when associated with AV, it presented regenerativepotential and angiogenic effect, with therapeutic potential for the treatment of grade I or II burns. Thus, obtaininga quick and complete recovery of the epithelial tissue of the cutaneous lining of all animals with a dressing containing 35% AV.

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INFLUENCE OF MIXED SOLVENT CONCENTRATION ON THE OBTAINMENT OF ELECTROSPUN PLA FIBERS

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Keywords: nanofibers; PLA; electrospinning; tissue engineering

Introduction and objective: PLA (Polylactic Acid) is a biodegradable thermoplastic biopolymer derived from renewable sources and is widely used in tissue engineering due to its biocompatibility and mechanical properties.For the electrospinning of PLA nanofibers, dichloromethane (DCM) was used as the organic solvent responsible for dissolving the PLA pellets, and dimethylformamide (DMF) was used to improve the electrical conductivity of the solution. Therefore, the overall objective of this study was to produce PLA fibers by mixing solvents at different concentrations of DCM and DMF and observe their influences on the morphological, physicochemical, and biological properties of electrospun PLA fibers.
Methodology: For fiber production, a PLA solution concentration of 10% (w/v) was established. Regarding the solvent concentration, proportions of 30/70, 50/50, and 70/30 (v/v) of DCM and DMF were used, with differentflow rates of 1, 1.5, and 2 mL/h. PLA was dissolved at 100°C for 60 minutes under constant stirring, followed by 24 hours of agitation at room temperature for complete dissolution. The distance between the infusion pumps and the collector was 17 cm, with a voltage of 14 kV and a collector rotation speed of 1400 rpm. The samples were characterized by scanning electron microscopy (SEM), wettability, and cytotoxicity.
Results and discussion: It were observed that increasing the concentration of DCM in the solution resulted in solution accumulation at the tip of the syringe. Therefore, with higher concentrations of DCM, it was necessary to pause the electrospinning process for cleaning. Increasing the flow rate resulted in the presence of beads dueto insufficient solvent evaporation during the journey from the spinning nozzle to the collector. Analyzing the distribution of fiber diameters, it was found that increasing the concentration of DCM led to an increase in fiberdiameter. The fibers made from the 30/70, 50/50, and 70/30 solutions with a flow rate of 1 mL/h presented the following average diameters (µm), respectively: 0.3855, 0.4305, and 1.608. Additionally, when examining the fiber diameter distribution, fibers electrospun from solution with a DCM and DMF ratio of 30/70 exhibited the most uniform fibers. All electrospun samples showed hydrophobic characteristics, with contact angles ranging from 127.3° to 140.8°. Cell viability tests were also conducted to determine the compound's toxicity, effectiveness, and safety. A viability of over 90% was obtained, indicating that the cells are in good condition, maintaining their structural integrity and cellular function.
Conclusions: It was possible to produce PLA fibers by electrospinning for all concentrations and flow rates, considering the influence of the concentration on the fiber diameter. The contact angle test confirmed the low wettability of PLA fibers, indicating a hydrophobic nature. Furthermore, cellular viability tests confirmed that PLAfibers didn't cause significant damage to cells or exhibit acute toxic effects. As future work, mechanical analyseswill be conducted, and the incorporation of hydroxyapatite will be explored to obtain biocomposite fibers.

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INFLUENCE OF nHA PARTICLES SIZE ON THE PROPERTIES OF 3D PRINTED PLA-BASED SCAFFOLDS

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Keywords: 3D printing; scaffold; PLA/nHA; bone tissue

Introduction and objective: The production of scaffolds through 3D printing technique has been presenting itselfas an alternative that allows the creation of a patterned structure able to act as an extracellular matrix, assistingin cell proliferation, differentiation and biosynthesis on their surface, while providing mechanical support. Research shows that the incorporation of bioceramics such as nanohydroxyapatite (nHA) in biopolymeric matrix such as PLA (Polylactic acid) enhance the biocompatibility, providing bioactivity, osteoconductive and osteoinductivity and improving in mechanic resistance [1,2]. This study aimed to develop and characterize PLA/nHA biocomposite scaffolds with a concentration of 10% nHA with different particle sizes using 3D printing.**Methodology:** First, biocomposite membranes were prepared by casting using PLA, dissolved in chloroform, and10 wt% nHA (obtained from the fish bone) with different particle sizes 9.86 nm and 34 nm, named AM1 and AM2, respectively. After obtaining the membranes, they were cut and loaded into the 3D printer to print the scaffolds. The scaffolds were printed at a temperature of 195 °C, fill of scaffold 90% and velocity of print 45 mm/min. Materials were characterized by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), compressive strength, cell viability, bioactivity in vitro and degradation in vitro.
Results and discussion: According to the DSC analysis, the addition of nHA did not significantly change the melting point of the material, which was approximately 152°C. The TGA analysis determined the residual mass of the biocomposite to be about 10.7%, equivalent to the added nHA, proving that the nHA particles were homogeneously dispersed in the material. SEM analysis of the scaffolds showed uniform filaments with nHA particles distributed along the surface. Regarding compressive strength, the average value of the tensions of thePLA, AM1 and AM2 samples were 15.84, 24.10 and 24.88MPa, respectively. In vitro bioactivity studies indicatedthat the biocomposite scaffolds showed bioactivity after 14 days, with the formation of globules and depositionof a crystalline layer, characteristic of apatite. A small variation in the weight of the scaffolds was detected in thedegradation test, since the degradation mechanism of PLA has a very slow. The cytotoxicity test, performed withmurine fibroblast cells, showed no cytotoxic effect of the scaffolds in the cells, obtaining approximately 100% viability in relation to the control well.
Conclusions: It was concluded that the size of the particles does not influence the thermal properties of the biocomposites, since there is no change in melting temperature. In addition, the mechanical properties were adequate for application in bone tissues. The addition of nHA increased the mechanical resistance and its bioactive capacity compared to pure PLA, improving its biocompatibility with bone tissues. Therefore, 3D-printedscaffolds are feasible for application in bone tissue.

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FUNCTIONALIZATION OF CLOISITE 30B® AND CLOISITE Na+® WITH CAPSAICIN MOLECULES FORBIOMEDICAL APPLICATIONS

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Keywords: Nanoclays, Capsicum, functionalization, biomedical.

Introduction and objective: Montmorillonite (MMT) clay is widely used in the biomedical field, as well as in tissueengineering, wound healing, and drug delivery. Cloisite 30B® is a clay from the MMT subclass that is modified withthe quaternary ammonium surfactant molecule known as MT2EtOH. Cloisite Na+® is considered a nanoclay of theMMT class, which contains Na^+ molecules within its structure. In this study, the functionalization of Cloisites withCapsaicin, an alkaloid naturally found in fruits of plants from the *Capsicum* family, was evaluated and analyzed using FTIR, DSC, and TG analysis methods.

Methodology: The functionalization was performed by agitating Cloisite in deionized water at a temperature of 37to 40°C for 15 minutes. After this process, 325 mg of diluted capsaicin in ethanol was incorporated, and the mixturewas stirred for 48 hours. Following this period, the clay samples were washed to remove residues using centrifugation. The samples were then dried at room temperature for 24 hours. FTIR, DSC, and TG analyses wereperformed on all samples.

Results and discussion: Based on the FTIR results, it is possible to observe that functionalization with capsaicin occurred for both clays, Cloisite 30B® and Cloisite Na+®. However, the Cloisite 30B® functionalization was less pronounced compared to the Cloisite Na+® spectrum. In the spectrum of Cloisite Na+®, characteristic bands of both the clay and capsaicin were observed: at 1040 cm^{-1} due to the stretching vibration of Si-O-Si in the clay; at 3315 cm^{-1} due to N-H stretching; at 2926 cm^{-1} and 2864 cm^{-1} due to C=O stretching, and at 1633 cm^{-1} and 1556 cm^{-1} due to aromatic C-C stretching, which are characteristic of capsaicin[1,2]. The DSC analysis revealed a shift in the melting temperature (T_m) of Cloisite 30B® functionalized with capsaicin to 175°C, despite not exhibiting the $T_m$of capsaicin at 55°C. This indicates that the functionalization was not highly pronounced, however, this shift suggests a modification of the material. On the other hand, Cloisite Na+® functionalized with capsaicin displayed both the T_m of capsaicin, around 55°C, and a shift in the T_m of Cloisite Na+®. The TG analysis demonstrated a displacement in the T_{onset} for both Cloisite 30B® and Cloisite Na+® with capsaicin, providing evidence of clay functionalization with the capsaicin molecule.

Conclusions: Based on the preliminary results obtained, both Cloisites were successfully functionalized with capsaicin, as evidenced by the modifications observed in the FTIR bands and the shift in the melting temperatureas indicated by the DSC analysis. The TG results also provided valuable insights for the study. It is worth mentioning,the outcomes demonstrated greater effectiveness in Cloisite Na+® functionalization by capsaicin through the conducted analysis. Thus, Cloisite Na+® proved to be a functionable material with potential for biomedical applications.

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ELECTROSPUN MEMBRANE OF POLY(L-co-D,L LACTIC ACID) AND NATURAL RUBBER CONTAINING COPAIBA OIL FOR ANTIMICROBIAL DRESSING DEVELOPMENT

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Keywords: PLDLA; biomaterials; natural extract; antibacteriostatic

Introduction and objective: The release systems of natural antimicrobial compounds, such as copaiba oil (CO), have gained prominence in the scientific community due to the growing concern about antibiotic resistance andits implications for public health. The electrospun devices function as efficient drug delivery systems, reducing side effects and increasing treatment effectiveness. Thus, the study aimed to evaluate the resulting effect of incorporating different concentrations of CO into a electrospun membrane composed of poly(L-co-D,L lactic acid)(PLDLA) and natural rubber (NR), with the goal of reducing systemic side effects and improving the efficacy of treating injured tissues.
Methodology: Membranes were prepared using separate solutions of 10% PLDLA (70:30) (w/vol.) and 1.5% NR in chloroform. These solutions were simultaneously electrospun using a DBM Eletrotech equipment system. Pure CO (*Copaifera officinalis L.*), obtained from DV-Manipulation Pharmacy and Natural Products, was dispersed in the electrospun membrane at concentrations of 25, 50, and 75% by dilution in an ethyl alcohol solution (70%) and Tween 20. The CO containing membranes were then dried at room temperature for 3 days and stored in a vacuum desiccator.
Results and discussion: The PLDLA/NR electrospun membrane, containing 25, 50, and 75% CO, underwent antimicrobial testing against *S. aureus* at 8 and 24 hours of culture. The CO exhibited bacteriostatic and antibacterial effects against *S. aureus*, due to antimicrobial properties [1]. The sample containing 75% CO showing the largest zone of inhibition. Scanning Electronic Microscopy confirmed that the membrane fibers hada morphology rich in recesses and pores, and the presence of CO prevented bacterial biofilm formation. The minimal incorporation of CO in the electrospun membrane caused a drastic decrease in the number of bacteria adhering to the membrane which provides a favorable microenvironment for microorganism growth [2]. The Crystal Violet test demonstrated strong bacterial inhibition in membranes with 50 and 75% CO. In these samples,the culture appeared more translucent, indicating a lower bacterial presence due to the release of oil concentrations into the bacterial culture medium. The percentage of swelling decreased with increasing CO concentration in the electrospun PLDLA/NR membrane, indicating that the addition of CO creates a safe environment for tissue recovery while acting as an antimicrobial agent.
Conclusions: The PLDLA/NR electrospun membrane has demonstrated promising antimicrobial properties with
the incorporation of CO, a desirable feature in wound dressings. The presence of CO also provided a physical barrier with prophylactic antimicrobial properties, making it a potential candidate for application in dressings, asit can prevent infections and contamination during the tissue healing process.

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OSTEOCONDRAL TISSUE REGENERATION PROMOTED BY ELASTIN-DERIVED HYDROGEL

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Keywords: osteochondral lesions; recombinant proteins; regenerative medicine**;** elastin

Introduction and objective: Osteochondral injuries involve the hyaline cartilage and subchondral bone of the joints. They are caused by various aetiologies, mechanical factors, recurrent trauma, overload, infections, degenerative diseases, among others, generating a health-socio-economic problem with a high impact on the population. This study aims to generate an experimental model of osteochondral injury in the knee of *New Zealand* rabbits, and to investigate whether a novel hydrogel (Hi) obtained by molecular biology techniques such as recombinant proteins of elastin, placed at the injury site, promotes the repair of the damaged tissue without undesirable effects.

Methodology: ELR polymers were produced as previously described in (10.1088/1758-5090/ab10a5). HRGD6- ELR consists of cell-adhesive RGD sequences, whereas DRIR-ELR contains sequences that are sensitive to the uPAenzyme. These click-modified ELRs were mixed to obtain the final hydrogel (**Hi**). Various techniques were employed for the physicochemical characterization of the hydrogels, including SEM, SDS-PAGE electrophoresis, and rheology. The cytocompatibility (**Cy**) of **Hi** was determined *in vitro* (bioassay, alamarBlue). 18 *New Zealand* rabbits were divided into three groups I, II, III (n=6). **I** (control), **II** and **III** underwent osteochondral knee injury surgery. **III** received at the site of the injury, **Hi** implant. Clinical, biochemical, magnetic resonance imaging **RMI**;and histopathological studies were performed**.**

Results and discussion: The microstructure of **Hi** shown an 80% porosity. *In vitro* degradation rates were studied using SDS-PAGE, and the complete polymer degradation after 48 hours was found crucial for the growth of cellsand their extracellular matrix generation. A complex elastic modulus of 1085 ± 145Pa was exhibited by the Hi hydrogel. **Cy** showed that **Hi** promote adherence and proliferation of HUVEC cells. There were no intergroup differences for the clinical surveys carried out during the 4 months of study. When performing biochemical studies, no significant intergroup differences were observed in values of white and red blood cells and platelets,hemoglobin levels, and serum ALAT and ASAT. **MRI** showed in II no cartilage regeneration, bone edema, hydrarthrosis and sclerosis subchondral in more than 50% of the samples. II showed cartilage regeneration in most of the samples and little development of subchondral sclerosis. Histopathological studies: II showed at thesite of the injury, an articular surface with hyaline cartilage of reduced thickness, less extracellular matrix and fewer chondrocytes, which were dispersed and formed incomplete isogenic groups. III showed at the site of thelesion that was implanted with HI, hyaline cartilage of increased thickness, with increased matrix and increased number of chondrocytes arranged in axial and coronal isogenic groups.

Conclusions:

The rate of hydrogel degradation is essential for biomedical applications, as the time required for tissue repair determines their effectiveness for a specific function. This study employs a slowly degrading Hi hydrogel that offers sufficient porosity for cell proliferation and mechanical properties that are appropriate for the regeneration of osteochondral tissues.

HIGH STRENGHT BIOMIMETIC SILK FIBROIN/α-TRICALCIUM PHOSPHATE BONE CEMENT WITH CHLORIDE-SUBSTITUTED HYDROXYAPATITE

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Keywords: tricalcium phosphate; silk fibroin; chloride-substituted hydroxyapatite; biomaterial.

Introduction and objective: Over the past decades, bone defects has been a rising factor leading to an increase in disability among patient and adversely affecting their quality of life. Large bone defects have limited inherentregenerative capacity, requiring surgical intervention for restoration [1]. As a result, there is considerable interest in the development of α-tricalcium phosphate-based cements as potential solutions for bone filling and regeneration applications. However, the mechanical properties of α-TCP cements are currently inadequate for many orthopedic applications [2]. The primary objective of this research is to develop a biomimetic α-TCP cementreinforced with non-dialyzed silk fibroin solutions.
Methodology: The CPCs were prepared by the manual mixture of a synthesized α-TCP powder with a non-dialyzed SF solution, which consists of dissolved SF in a $CaCl_2$:EtOH:H_2O ternary solution, in order to obtain 25wt% of liquid phase in the cement. This process was performed for SF solutions with different concentrations. The resulting paste was transferred to different molds for hardening at 37°C in a 100% relative humidity chamber. The developed CPCs were characterized by x-ray diffraction, x-ray fluorescence and Fourier transform infrared spectroscopy, and had their porosity, compressive strength, fracture toughness, microstructure and NIH-3T3 cellviability assessed.
Results and discussion: CPCs with SF reinforcement exhibit a facilitated transformation of the α-TCP starting material to apatite products; moreover, the presence of chloride ions in the SF non-dialyzed solution promptedthe formation of a biphasic material consisting of CDHA and chloride-substituted hydroxyapatite (HAp-Cl). The presence of HAp-Cl has the potential to enhance the osteoconductivity of the final material. Regarding the mechanical properties of the obtained CPCs, reinforced samples notably demonstrated a significant increase in fracture toughness and compressive strength. Samples with 0.38wt% of SF demonstrated an increase of 209% of the compressive strength and 396wt% of the fracture toughness, while samples with 0.50wt% SF demonstrated increases of 182% and 450% in these respective properties, despite having porosities in the order of 31%. Furthermore, the microstructure of all SF-reinforced samples exhibited a more biomimetic structure with smaller needle-like crystals when compared to the control sample, potentially contributing to
the material's reinforcement. Most importantly, the composition of the reinforced samples did not adversely affect the cytotoxicity of the CPCs and even improved the cell viability compared to the control CPC.
Conclusions: This study successfully obtained biomimetic CPCs with mechanical reinforcement through theaddition of SF. The obtained properties suggest an effective bonding between SF and the calcium phosphates,and the microstructure demonstrated the high capacity of SF to regulate and orient the crystal growth of calciumphosphates. The interaction of the organic and inorganic components of the developed CPCs led to a materialwith potential to be further studied as a suitable material for bone regeneration.

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PRODUCTION AND CHARACTERIZATION OF A BIOINK CONTAINING DECELLULARIZED SPINAL CORD TISSUE AND AN ELECTRICAL CONDUCTIVE POLYMER FOR 3D BIOPRINTING

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Keywords: Decellularization; 3D bioprinting; Bioink; Skin.

Introduction and objective: Skin injuries are still a major public health problem. Even though skin has good intrinsic regeneration capacity, larger and deeper wounds that reach the dermis pose a more complex issue [1].This type of injury can lead to complications, such as infections and even death. Current treatments are still limited and easily-available alternatives are necessary. In this context, 3D bioprinting is a promising candidate since a bioprinted material can restore skin's barrier functions and integrate with the patients tissue [2]. The aimof this study was to produce a bioink using lyophilized rat Decellularized Skin (DS) for 3D bioprinting.

Methodology: Rat skin was decellularized using three protocols. Genomic DNA was quantified and histological slides were stained with H&E, DAPI or Masson's Trichrome. The tissue was freeze-dried and combined with alginate, gelatin and stem cells to produce different bioinks with concentrations of 1.5% or 3% DS, 7% gelatin, 3% or 4% alginate and $1.0X10^6$ cells/mL. Bioink swelling was evaluated for 4 weeks. Cell viability was analysed using live/dead assay. Cell proliferation was analysed using immmunofluorescence. Rheological characterizationwas performed using a rheometer with the Peltier equipment. Hydrogel SEM images were acquired using an electronic microscope.

Results and discussion: DNA quantification showed no difference in gDNA concentration in the three protocols and histological slides indicated a lower cell presence in the decellularized tissue in comparison to native skin. For that reason the 9 hours protocol was chosen. Masson's Trichrome staining showed abundant cytoplasm presence in the native tissue whereas the decellularized samples presented a high collagen staining. Bioink swelling was evaluated by measuring the tissue area and only the bioink composed of 1.5% DS, 3% alginate and7% gelatin was able to maintain the construct integrity for 4 weeks and was chosen to continue the tests. Live/Dead assay was done 7 days after bioprinting and showed a higher viability in the biomaterial when compared to the control. The bioprinted material was able to maintain cell proliferation activity during 14 days.The hydrogel presented shear thinning behaviour and a constant viscosity even with different concentrations. SEM images showed that the biomaterial presented a porous tri-dimensional structure.

Conclusions: As described above, the decellularization protocol established was able to significantly reduce the DNA content. 1,5% DS, 3% alginate and 7% gelatin hydrogel had better mechanical qualities and preserved structural integrity, therefore, was selected for *in vitro* testing. The bioprinted material allowed for a long-term cell viability and proliferation. Thus, this bioink, containing decellularized skin, could be a viable and easily- available alternative for regeneration of skin diseases through 3D bioprinting.

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PARTIALLY AND FULLY HYDROLYSED POLYVINYL ALCOHOL CROSSLINKED WITH HEXAMETHYLENEDIISOCYANATE AS A BONE SCAFFOLD

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Keywords: PVA; hexamethylene diisocyanate; biomaterials; crosslinking, bone tissue engineering

Introduction and objective: Bone diseases incidence have increased in recent years[1]. New bone scaffolds are needed for bone tissue engineering for both orthopaedic and dentistry applications. These biomaterials should be biocompatible, allow cell colonization, have adequate mechanical properties and a porous structure with a pore size distribution, which allows cell and nutrients migration besides metabolites transfusion[2]. Polyurethanes are being used in the biomedical field. The objective is to create a bone scaffold by crosslinking polyvinyl alcohol (PVA) with different acetylation degrees with hexamethylene diisocyanate (HDI)monomers and oligomers to form a porous structure with no cytotoxicity and capable of cell adhesion and colonization.

Methodology: A 15% solution of partially hydrolysed (PH) or fully hydrolysed (FH) PVA was prepared in hot water. Then it was placed in adequate moulds and lyophilized. Afterwards, it was crosslinked with HDI, coming from two different origins: pure reagent from Sigma (monomer), and a paint hardener from a national brand, composed of HDI oligomers. The crosslinking was performed at 60°C under inert conditions in a proportion OH: NCO 1:1. To calculate the active isocyanate groups present in the paint hardener, a back titrationin non-aqueous media was made. The materials were physiochemically characterized and their cytotoxicity wasevaluated.

Results and discussion: Polyurethane bonds were determined by IR spectroscopy, studying their characteristic peaks**.** SEM/EDS was used to study the morphology and nitrogen content. The scaffolds obtained after crosslinking with HDI or HDI- paint hardener exhibited different characteristics. In the first case, a cellular structure was distinguished while a rough fractured surface was present in the other samples. Nitrogencontent was higher for HDI-crosslinked materials in comparison to materials obtained with paint hardener (for both FH and PH PVA). These results were in concordance with the gel content assay. TGA-DTGA showed PVA-PHmaterials crosslinked with HDI had higher degradation temperatures indicating a thermal stabilization of the polymer. On the contrary, PVA-FH materials crosslinked with HDI showed lower temperatures due to lack of hydrogen bonding stabilization. DSC assays were also performed. Porosity was studied by mercury porosimetry,being the accessible porosity around 30%, with an average pore size of 189.27 nm and 4.9 µm for PVA-HDI and PVA-paint hardener, respectively. Biological reactivity was studied using MC3T3 fibroblasts after 48h, indicatingthat none of the materials present toxicity. Cell colonization was also assayed measuring LDH enzyme activities showing non-significant differences between the four materials but low proliferation after 3 days with respect to a control.

Conclusions: PVA was successfully crosslinked with both HDI sources. It shows differences in the final characteristics when the reagent is composed by monomers or oligomers. Differences in the stability inwater were observed between PVA-PH and FH, resulting that PVA-FH is a less stable material, especially when itis crosslinked with HDI-based paint hardener. None of the materials were toxic for fibroblast cells, however the colonization of the materials is being improved by a collagen type I coating with promising results.

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SPINNABILITY OF POLYCAPROLACTONE FIBERS LOADED WITH STRONTIUM-MODIFIEDHYDROXYAPATITE

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Keywords: polycaprolactone; solution blow spinning; hydroxyapatite; nanofibers

Introduction and objective: Polycaprolactone (PCL) Fibrous mats can mimic the fibrillar structure of the extracellular matrix of mammalian tissues [1]. Nevertheless, its low bioactivity can be a barrier to its applicationin tissue engineering. The addition of Strontium substituted Hydroxyapatite (HASr) in PCL fibers can be an easy and efficient alternative to enhance the potential of fibers to lead to bone tissue regeneration[2]. This work aimed to produce and characterize fibrous mats of PCL and strontium-substituted hydroxyapatite (HASr) produced by solution blow spinning (SBS)

Methodology: PCL (Sigma Aldrich) 12% solutions (w/v in acetic acid) were prepared at 45°C and stirred until PCL solubilization, and 1%, 10%, or 30% (w/w) of HASr was added. The viscosity and surface tension of each solutionwas analyzed. Solutions were spun with a flow rate of 6 mL/h, air pressure of 10 psi, and work distance of 30 cm.Fiber morphology was evaluated by SEM, and image processing was performed using the Size Meter 1.1 software. Physical and chemical behavior was assessed, and fiber swelling was evaluated by soaking the mats insaline solution for 14 days.

Results and discussion: The addition of 30% HASr considerably increases the viscosity of the PCL solution while reducing the surface tension compared to pure PCL. The fibers are randomly oriented, and some defects knownas beads were observed for all HASr concentrations. The measurement of fiber diameters shows that PCL/HASr 0, PCL/HASr 1%, PCL/HASr 10%, and PCL/HASr 30% fibers were obtained with a mean diameter of 0.6129 ± 0.3023
µm, 0.566 ± 0.362 µm, 0.6129 ± 0.3939 µm, and 0.489 ± 0.211 µm respectively. The addition of higher concentrations of hydroxyapatite significantly alters the swelling capacity of the blankets. PCL/HASr 0 fibers absorbed about 260% of their weight in water, while fibers with 10 and 30% absorbed 35% and 56% of their weight in water, respectively. This fact may be correlated with the formation of crosslinks due to the interactionof HASr with the PCL chains. A preliminary biological study shows that all mats are not cytotoxic for cells *in vitro*.**Conclusions:** The results show that is possible to produce fiber mats even with a higher amount of HASr, withoutcompromising the structure of fibers. The preliminary biological response shows that this material has great potential to enhance bone tissue regeneration.

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PLA-BIOAlSr GLASS COMPOSITE AS A CANDIDATE FOR BONE AND TENDINOUS REGENERATION APPLICATIONS

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Keywords: biomaterials; tissue engineering; poly(lactic acid); bioactive glasses

Introduction and objective: Poly(lactic acid) (PLA) is currently one of the most promising biodegradable and biocompatible materials once it can be produced from renewable resources and used in packaging, biomedical and tissue engineering applications. In the past decade, composite materials combining biodegradable polymers with inorganic materials such as bioactive glasses are being studied showing remarkable improvements mostly in mechanical and thermal properties. Previous studies conducted by our research group proposed a bioactive glass composition containing strontium and alumina, BioAlSr, ensuring osteointegration and superior mechanical properties [1]. Here, a composite film of PLA and BioAlSr was proposed for bone tissue regeneration.

Methodology: To produce the composite films chloroform was used as cast solvent. The PLA was dissolved in chloroform at 3,3g/ml and the BioAlSr particles was added in ratios of 15, 30, 50 and 70 wt%. The glass particles were submitted to ultrasonic treatment to permit more efficient dispersion in the PLA matrix. The obtained films were analysed for their microstructure with X-ray diffraction and Fourier transform infrared analysis, homogeneity by polarized light microscopy and and in vitro cytotoxicity by NCTC clone 929 cell line.

Results and discussion: All XRD spectra showed predominantly amorphous state of the composite films. To examine the existence and type of interfacial interaction in the composites, FT-IR experiments were performed and compared with pure PLA and BioAlSr. The regions of interest were 1780 and 1680 cm^{-1} for the C=O stretch, and 3600–3000 cm^{-1} for the O–H stretch from PLA known bands [2] and the region of 800-1300 cm^{-1} corresponded to the stretching vibrations of the silica of the bioactive glass was analysed [1]. PLA characteristic bands were predominant even in samples containing 50 and 70 wt% of BioAlSr. The dispersive effect of the glass particles in the PLA matrix was also evaluated by polarized light microscopy and the results demonstrated an adequate homogenization. Also, cytotoxicity and cell viability obtained by using the NCTC clone 929 cell line did not show any significant loss of cell viability or cytotoxicity.

Conclusions: Preliminary results of the proposed study indicate the obtention of a homogeneous composite film with adequate interaction between the matrix and the dispersed material, preserving the microstructure of both materials. Furthermore, the material obtained did not show cytotoxicity, indicating that it is a promising alternative for the application of bone and tendinous regeneration.

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COLLAGEN COMPOSITES WITH SELENIUM NANOPARTICLES AS DRESSINGS FOR CHRONIC WOUNDS

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Keywords: Selenium nanoparticles; Composites; Dressing, Cytocompatibility; Antimicrobial activity

Introduction and objective: Within the health area, an important topic in which the use of nanoparticles is sought to be applied is in the care of chronic wounds such as those produced in diabetic people, which are characterized by not having good healing and by suffering repeated infections, making it necessary to implement dressings. Selenium nanoparticles (SeNp) have shown antimicrobial, antioxidant and anti-inflammatory activities, so their application in this area would be promising [1]. In this work, the chemical synthesis of SeNp with its corresponding characterization, both in solution and in collagen gels so that they can be applied as dermal dressings in the near future.
Methodology: SeNp were synthesized using cysteine as reducing agent and stabilizer and Na_2SeO_3 as precursor. For characterization, UV-Vis spectroscopy was used. Morphology and size were observed by TEM and DLS. Antimicrobial and cytocompatibility assays were done. Collagen composites were synthesized in different ways:**1-** already synthesize SeNp were included during gelation (mixture) **2-** they were prepared in situ by immersion of the gel in cysteine and Na_2SeO_3 solutions **3-** similar to 2, but fixing cysteine to collagen with glutaraldehyde. FT-IR and ICP-OES were used for characterization as well as SEM and EDS. Antimicrobial, antioxidant and cytocompatibility assays were also carried out.
Results and discussion: SeNp synthesis had a yield of 99.3%. They had a homogeneous rounded morphology and a size of 142 ± 44 nm by TEM images, and 172 ± 3 nm by DLS. When analyzing its cytocompatibility with 3T3 fibroblasts, the LC50 was 167 ppm. On the other hand, antibiograms showed a large inhibition zone using concentrations above 25 ppm. However, its minimum bactericidal concentration could not be determined up to 300 ppm. Red collagen composites were obtained evidencing the presence of SeNp, also corroborated by SEM noting Np of sizes of 116± 46 nm, 200± 48 nm, 110± 17 nm, and 255± 121 nm for Col Glut-SeNp gels (cis 0 .1), Col-Glut-SeNp (cis 0.5), Col-SeNp (cis 0.1) and Col-SeNp (cis 0.5), respectively. Se concentrations were 149±24, 284±40, 167±17, 433±89 and 158±19 for mixture composites. Mixture composites did not show cytotoxicity, as well as composites with Np made *in situ* with 0.5M cysteine (either crosslinked or not with glutaraldehyde). Oppositely, composites made *in situ* with 0.1 M cysteine, both cross-linked or not, showed a decrease in cell viability. Regarding the antimicrobial activity, it was seen that composites crosslinked with glutaraldehyde had a greater antimicrobial activity against *S.aureus* with respect to gels without crosslinking and the latter, in turn, showed greater activity than mixed gels.
Conclusions: SeNp had a LC50 of 167ppm, being less toxic than previously tested AgNp (46ppm). No activity against *S. aureus* was observed in liquid medium, but inhibition zones were observed in antibiograms, indicating that their antimicrobial activity is moderate to weak. By incorporating Np in composites, antimicrobial activity increased. On the other hand, cysteine could function as a protector towards cells, as at higher concentrations of cysteine, cell survival was greater. Moreover, Np synthesized with more cysteine showed larger sizes which also affects cell viability.

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DEVELOPMENT AND CHARACTERIZATION OF COLLAGEN TYPE I BIOCOMPOSITES REINFORCED WITH 2D NANOMINERLS FOR BIOMEDICAL APPLICATIONS

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Keywords: biocomposites; collagen; talc; 2D materials

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Introduction and objective: The development of biological devices capable of regenerating or repairing damaged tissue without the need to replace it is possible by combining body cells with highly porous materials. Scaffolds, 3D structures used as supportor reinforcement, promote cell proliferation guiding the growth of new tissues. In this research, a biocomposite constituted by a matrix of collagen type I from bovine tendon and nanominerals, talc and mica, were developed. Talc and mica are phyllosilicate minerals having lamellar structures interconnected by van Der Waals interactions,making them a source of 2D materials.
Methodology: We obtained Bovine type I collagen following an extraction protocol [1]. For the production of nanostructures of talc and mica, the exfoliation method in liquid phase was used. Talc and mica were exfoliated, with talc in butanoneand mica in ethanol, both at a concentration of 6mg/ml (milligrams of powder per milliliter of solvent). A solutioncontaining talc and mica was kept in a bath in a flask and kept in an ultrasonic bath for 15 hours [8]. Butanone andethanol were evaporated and nanostructures supported for the production of nanobiocomposites.
Results and discussion: Preliminary AFM results reveal that talc and mica flakes have an average thickness of less than 20 nm. AFM furtherenabled us to assess the dispersion of nanotalc within the polymeric matrix. SEM images validate the expected morphology of collagen fibers following the neutralization process. EDS analysis has substantiated the presence of magnesium, one of the constituents of talc, within the polymeric matrix. Furthermore, spectroscopic techniquessuch as FTIR and Raman have substantiated that the liquid-phase exfoliation process for obtaining nanostructuresdoes not alter the internal crystalline structure of phyllosilicates. The in vitro biological assays conducted on nanostructured talc and mica have yielded promising results regarding their viability. These assays encompassed a range of tests, including cell viability assays, cytotoxicity assessments, and cell proliferation studies. The findings indicate that the nanostructured forms of talc and mica exhibit favorable biocompatibility and cellular response profiles.
Conclusions: Based on the presented data, we infer that the obtained nanostructures have the desired thickness for the production of nanobiocomposites. Through in vitro and in vivo studies, the possible clinical applications of the developed scaffolds will be analyzed.

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THE PHARMACOLOGICAL EFFECTS OF ARNICA EXTRACT INCORPORATED INTO PVA [POLYVINYL ALCOHOL)] IN A DELIVERY SYSTEM ASSOCIATED WITH LOW-INTENSITYLASER

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Keywords: burn; biomaterial; PVA; low-intensity laser.

Introduction and objective: The skin is the organ most affected by 2nd degree burns because the damage is more extensive and worrying. The use of a biomaterial can help in the cellular repair process with better prognosis, low-cost and better acessibility. Arnica extract has proven to have anti-inflammatory and analgesic properties in skin lesions treatments and laser therapy is another alternative therapy in the biomodulation of theinflammatory process in burns. Evaluate the pharmacological effects of the Arnica extract incorporated into PVA (polyvinyl alcohol) in a modified release system associated with low intensity laser on 2nd degree burns.

Methodology: Pure PVA (PVA) or PVA membranes with 5% Arnica extract (PVA+A) were obtained and characterized (physicochemical methods) for application in skin lesions. Through *in vivo* studies the effects of the bandage of PVA and PVA+A membranes, with or without the application of low intensity laser (L), on 2nd degree burns in Wistar rats that enabled histological analysis (Hematoxylin-eosin and Masson Trichrome) and immunohistochemistry [markers: CD68, alpha smooth muscle actin (a-SMA) and fibronectin] in the diferente experimental groups.

Results and discussion: The thermogravimetry curve showed that the addition of Arnica to the PVA matrix did not alter the thermal resistance of the polymer; the differential exploratory Calorimetry demonstrated that the addition of Arnica increased subtly the value of the crystallinity of the PVA+A sample when compared to the PVA one; in the traction mechanical test, after the Arnica addition there was a reduction in the value of the elastic modulus when compared to PVA; in the Fourier Transform Infrared Spectroscopy there was no interaction between the Arnica molecules and the PVA chains; in the swelling test the presence of Arnica reduced the capacity of membrane swelling. The controlled release profile of Arnica showed a rapid release to the environment in up to 9h and sustained release until the end of the test. In the *in vivo* studies, regarding tissue repair, the PVA+A+L group identified more collagen fibers than in the control group ($p<0.05$); with immunohistochemical markers the highlight was with a-SMA where PVA and PVA+L groups increased myofibroblasts ($p<0.05$). Both the increase in the number of collagen fibers and myofibroblasts accelerate the healing process, thus reducing the time of tissue repair.

Conclusions: The incorporation of Arnica extract to PVA was biocompatible and with sustained release of the active effectively, being a promising bandage option for skin lesions. Through this study we also reiterate that low-intensity laser therapy with or without Arnica was effective in accelerating the healing process due to its potential biomodulatory effect, improving inflammatory aspects, as well as the conditioning of collagen fibers, promoting rapid healing in skin lesions.

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IN VITRO STUDY OF MANGANESE (Mn^{2+}) ASSOCIATED WITH HYALURONIC ACID ONTHECYTOTOXICITY OF *BOTHROPS JARARACUSSU* VENOM

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Keywords: manganese; hyaluronic acid; Bothrops; cytotoxicity.

Introduction and objective: Ophidian accidents pose a major public health concern, especially in rural areas with limited medical access. In Brazil, Elapidae and Viperidae families account for most envenomation cases, notably Bothrops genus. Lack of complementary treatments to antivenom and limited therapies may lead to significant victim consequences. Bivalent ions like manganese (Mn^{2+}) demonstrate protective effects against local envenomation effects, while hyaluronic acid (HA), a crucial extracellular matrix (ECM) component, regulates cell processes. This study aims to assess the in vitro therapeutic potential of manganese associated with AH for modified release against the cytotoxic action of Bothrops jararacussu venom (Bjssu).

Methodology: The characterization of Mn^{2+} AH and release assay were performed. MTT assays will be performedto evaluate cytotoxicity, using cell lines of rat myoblasts with different concentrations of Mn^{2+}, Bjssu, AH, aloneor in different combinations, such as: Mn^{2+} + Bjssu, Mn^{2+} + AH, Bjssu + AH, Mn^{2+} + Bjssu + AH. These assays will beperformed at 1, 2, 3, 6, 12 and 24 h. A comparative morphological evaluation will be performed with hematoxylin-eosin and rapid panopticon in the different protocols.

Results and discussion: The study aimed to determine safe concentrations of Mn^{2+} to avoid acidification of the culture medium and investigate its impact on cellular growth. Interestingly, treatment with Mn2+ demonstrated significant cellular growth promotion across most concentrations and time points, except during the 3-hour period. The tested concentrations of Mn^{2+} (ranging from 0.0075 to 0.12 g/ml) exhibited promising results, with no statistically significant losses observed, except for the 3-hour assay, where limited mortality, below 20% compared to the control group, was noted [1]. These findings indicate that Mn^{2+} can be safely utilized within the tested concentrations for further research, offering potential applications in ophidian accident treatment. Furthermore, the viability assay with hyaluronic acid revealed positive outcomes across a range of concentrations(from 75 to 2500 µg/ml), consistent with the existing literature [2]. The stability and functionality of hyaluronic acid within the tested range further emphasize its potential as a valuable component for therapeutic interventions.

Conclusions: Overall, no relevant physical or chemical alterations in both Mn^{2+} and HA underlines their potential use in further studies related to the treatment of ophidian accidents. These promising findings provide a solid foundation for exploring the potential therapeutic benefits of Mn^{2+} and HA in addressing snakebite-related issuesin the future. It's important to note that these results were obtained in vitro, and further research, including in vivo studies, will be crucial to confirming their safety and efficacy in practical applications.

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PHARMACOLOGICAL EFFECTS OF TRIAMCINOLONE ASSOCIATED WITH SURGICAL ADHESIVE ON CUTANEOUS WOUND HEALING IN WISTAR RATS

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Keywords: biomaterials; corticosteroid; 2-octyl-cyanoacrylate.

Introduction and objective: The surgical adhesive 2-octyl-cyanoacrylate is widely used for the closure of cutaneous surgical wounds. However, side effects such as contact dermatitis, dehiscence, and scar alterations are common and caused by the polymer itself. Exogenous corticosteroids are widely used for their anti- inflammatory and immunomodulatory properties [1). The resulting effect is a healing process with less collagen deposition, a smaller amount of granulation tissue, and fewer inflammatory cells [2}. The aim was to evaluate macroscopically and microscopically the pharmacological effects of triamcinolone acetonide (TA) incorporated into a commercial surgical adhesive (2-octyl-cyanoacrylate) on the initial phase of the surgical wound healing process in Wistar rats.

Methodology: The corticosteroid TA was added to the surgical adhesive (2-octyl-cyanoacrylate) and characterized physiochemically. Through in vivo studies, the effects of the healing process were assessed by using either the isolated adhesive or the compound in the same rat, enabling a controlled study with macro and microscopic analysis, including histology and immunohistochemistry, of the healing process in different incisions made on the back of each group on the 7th and 14th day post-surgery, respectively.

Results and discussion: The modified release assay showed drug release from the adhesive at concentrations of 5, 10 and 20mg/ml, with the latter showing a more linear release pattern up to 120h. Thermogravimetric analysis (TG) demonstrated that the addition of TA to the adhesive did not alter the thermal resistance of the polymer. Differential Scanning Calorimetry (DSC) showed that the addition of TA at various concentrations did not alter the adhesive's melting point to cause instability; thus, they can co-exist safely. However, Scanning Electron Microscopy (SEM) revealed a loss of homogeneity in the adhesive due to drug incorporation. Fourier Transform Infrared Spectroscopy (FTIR) indicated no interaction between the AT molecules and 2-octyl- cyanoacrylate, but it identified the presence of the drug at a concentration of 20mg/ml, which was chosen forthe in vivo phase. Regarding the macroscopic evaluation, there was no difference in healing or adhesion between the control and compound groups, only the presence of small particles in the lesion where thecompound was used. Histological analysis with hematoxylin-eosin staining and immunohistochemistry for tissue repair is still under analysis.

Conclusion: The incorporation of TA into the surgical adhesive did not alter its physicochemical properties. Therewas no interaction between the molecules; however, drug incorporation affected its homogeneity. The modified release assay showed the presence of the drug up to 120h in a linear manner at 20mg/ml. As for the microscopictissue repair process with histological and histochemical analyses, the results are awaited, expecting a reduced initial inflammatory response, minimizing the side effects of the polymer and scar alterations.

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PIPEq-PUC/SP

STUDY OF BIORESORBABLE POLYMERS THERMAL STABILITY FOR FIBER PRODUCTION

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Keywords: Poly(ε-caprolactone), Bioresorbable polymer, Gel permeation chromatography.

Introduction and objective: Melt Electrospinning Writing (MEW) and Fused Deposition Modelling (FDM) are additive manufacturing techniques that involve the deposition of polymer fibers to create 3D- structures. Both use a heated nozzle to extrude and deposit the molten polymer, but MEW also applies an electric field [1]. Previous studies on traditional electrospinning of poly(ε-caprolactone) (PCL) without heating showed polymer degradation [2]. Understanding the thermal stability of the polymer during its processing is of great importance. The aim of this work is to analyze changes in relative molecular weight distribution and polydispersities of PCL in different heating cycles using gel permeation chromatography (GPC).
Methodology: Samples of PCL (Mn ~ 45000 Da, Sigma-Aldrich) were melted at 90°C for 0, 1, 2, 3, 4, 8 and 16 hours. Melt PCL was processed by melt-electrospinning at 140°C with an electric field of 12 kV for 1-3 cycles. Samples from the different heating cycles were analysed by gel permeation chromatography in triplicates. GPC assays were performed at 25°C using a tetrahydrofuran mobile phase. Columns were provided by Shim-Pac (Shimadzu). Results were analyse by *Prominence* software.
Results and discussion: Although, differences in apparent molecular weights and polydispersities were observed at 0, 1, 2, 3, 8 and 16 hours of heating at 90°C, it is believe these correspond to the variability of the characterization technique. No significant difference ($p>0.05$) were observed when number average molecular weight (Mn) were analyzed for those heating cycles compared with unheated sample. However, a significant difference ($p>0.05$) at Mn was seen for the simple heated during 4 hours a 90°C. Besides, a decrease in molecular weights of samples processed by MEW at 140°C was observed compared to those only treated by heating at 90°C and the unheated sample ($p>0.05$). These results, indicate some degree of degradation in PCL chains during successive processing cycles, this behavior was also observed for solution electrospinning processing [2]. A synergistic effect due to melt processing and the high voltage electrical field applied to the polymer could be the cause of the observed degradation.
Conclusions: The variability of the characterization technique was determined by GPC analysis. Thermal degradation is an important aspect to consider when multiple heating cycles are implemented. Additionally, understanding the behavior of the polymer under an applied electric field is crucial. This becomes particularly relevant in the production of fibers using the MEW technique, as there is a dual contribution to degradation. However, due to the difference in processing temperature with respect to heating temperature, the contributions cannot be separated.

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RHEOLOGICAL CHARACTERIZATION OF SILK FIBROIN/PLDLA/SIMVASTATIN-BASED GEL FOR 3D PRINTING

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Keywords: 3D printing; biomaterials; bone tissue; rheology; silk fibroin; engineering; simvastatin

Introduction and objective: 3D Printing emerged to revolutionize Bone Tissue Engineering, combining biomaterials and osteogenic molecules such as simvastatin (SIM) that mimic bone tissue [1,2]. Silk fibroin (SF) isa promising biomaterial due to its mechanical and biological properties [1]. However, controlling its rheologicalprofile for 3D printing is a challenge. The addition of other polymers has studied to control the viscosity of the FS solution, guaranteeing the reproducibility and quality of the printed structure. The objective of this work is to study the rheological properties of the gel based on FS/PLDLA/SIM, for 3D printing of the scaffold, aiming at theregeneration of bone defects.

Methodology: The FS/PLDLA solutions were analyzed in a Rheometer DHR-2, TA Instruments at different concentrations, performing the following tests: viscosity curves as a function of shear rate (0.01 to 1000 s-1) in steady state; amplitude and frequency oscillatory tests (Small Amplitude Oscillatory Shear- SAOS), obtaining the loss (G'') and storage (G') modules; thixotropy test to evaluate the recovery of gel viscosity as a function of time(s). Once the appropriate formulation was chosen, scaffolds were printed on the Biotechnology Solutions 3D Printer (OctopusTM) in a grid pattern and reticulated in 70% ethyl alcohol.

Results and discussion: Among the solutions tested, the FS 15.0%/PLDLA 7% formulation showed an accentuated pseudoplastic region at high shear rates. In the frequency test (SAOS), the G'>G'' modules represent a typicalgel behavior, important to keep the scaffold structure intact, in addition to the viscosity being fully recovered during the thixotropy test. Assays with additions of simvastatin at concentrations of 0.1%; 0.25%; 0.5%; 1.0% and 2.0% did not significantly alter the behavior of the FS 15.0%/PLDLA 7.0% formulation.

Conclusions: The rheological tests showed that the chosen formulation met the criteria of pseudoplasticity, viscoelasticity and thixotropy, important for the 3D printing process. Scanning Electron Microscope (SEM), Infrared Spectroscopy (IR), TGA and DSC will characterize the printed framework.

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DEVELOPMENT OF A COLLAGEN AND CHITOSAN BIOINK

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Keywords: collagen, chitosan, 3D bioprinting, fibroblast bioink

Introduction and objective: The development of bioinks for 3D bioprinting has been extensively researched, given the technique's relevance in tissue engineering. While collagen and chitosan hydrogels are widely employed for designing scaffolds, their combined use as a potential bioink is rarely reported [1]. In fact, their poor mechanical performance in comparison to other hydrogel formulations, as well as their dissolution at acidicpH, pose drawbacks for this application. Considering the importance of collagen in tissue regeneration and the benefits of chitosan [2], our goal is to create a printable ink from these biopolymers, that allows for the addition of cells at neutral pH.

Methodology: Type I collagen from rat tail, and low molecular weight chitosan were dissolved together at 1.25% w/v each in 0.05 M acetic acid. To increase pH, nebulisation with NaHCO3 0.8 M was employed, tunning the times according the ink volume, as well as the rests between steps, measuring pH each time. Ink features were analysed by rheology. Printability was determined using an extrusion 3D bioprinter (LIFE SI, Argentina), through filament width and gap areas. We assessed cellular viability by printing the ink containing DMEM (added 10x) and 1.105 fibroblasts/mL. Cell viability was determined using confocal microscopy and a LIVE/DEAD kit.

Results and discussion: The collagen and chitosan blends had a pH of 5.30-5.40 and an apparent viscosity of 285 Pa.s at 0.015 s-1, determined by flow sweeps. By employing nebulisation in 3-4 steps separated each one by 10 minutes of rest, we achieved mixtures with a pH of 6.80-6.90. We intentionally avoided reaching a pH of 7.00 due to the pH-dependent gelation of chitosan. In addition to a pH increase, these nebulised inks showed an increase in viscosity, reaching 24.300 Pa.s on day 1 post-nebulisation. The pH remained stable over the following5 days, but the viscosity continued increasing. So, for the assessment as a possible ink, we decided to evaluate italways on day 1 after nebulisation. Regarding printability, the ink showed a tendency to spread after extrusion with a spreading ratio (measured filament width/needle diameter) of 2.8 in the first minutes; this condition affected the gap areas in grid designs.

The fibroblasts-laden bioink was extruded at an estimated shear rate of 10 s-1. We printed solid circular shapesthat were placed in a 24-wells plate under sterility conditions. By culture in complete DMEM at 37 C and CO_2 atmosphere, cell viability was estimated at days 1, 7 and 14 after extrusion. By image analysis we estimated theviability to be > 80% in the three times.

Conclusions: Due to collagen and chitosan properties, pH neutralisation was a mandatory step to include cells making up a bioink. In this sense, we developed a mild method to obtain a homogeneous neutral ink. Medical needs for soft tissue, such as skin regeneration, could benefit from such a bioink. Further research is being conducted to improve printability, by adding crosslinkers or a third polymer, as well as to explore the properties of the produced scaffolds.

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DEVELOPMENT OF BIOCOMPOSITE MEMBRANES OF BACTERIAL CELLULOSE ANDHYDROXYAPATITE FOR APPLICATION IN BONE TISSUE

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Keywords: bacterial cellulose; hydroxyapatite; biocomposite; bone tissue

Introduction and objective: Cellulose is a biopolymer from renewable sources, biocompatible and biodegradable. Bacterial cellulose (BC) is a natural polymer produced by specific bacteria. Its properties differ from vegetable cellulose, due to the high (purity, crystallinity, and water content retention capacity) [1]. Due tothese properties, it has been investigated as a biomaterial for the regeneration of bone tissue. Although, a BC has advantages, it has low bioactivity, being necessary to be associated with another material, an example of hydroxyapatite (HA) with excellent biocompatibility and osteoconductive capacity [2]. This study aimed to develop and characterize the BC/HA biocomposite for biomedical applications.

Methodology: BC was produced by static cultivation of the *Glucanoacetobacter hansenii* strain (ATCC23769) for14 days. After this period, the membranes obtained were purified using an alkaline treatment. High purity HA was synthesized by solution combustion. The biocomposite membranes were prepared by casting. The BC was ground and mixed with HA at concentrations of 0.2 and 0.5g wt% of HA, named BC/0.2HA and BC/0.5HA, respectively. Then the structures were dried at 37°C for 24 hours and characterized by SEM, FTIR, wettability, water solubility, and swelling. ANOVA and Tukey's post-test determined the analysis of statistical significance.

Results and discussion: SEM analysis showed HA particles dispersed along the BC matrix. The BC pure presented characteristic vibrational modes. For BC/0.5HA, absorption bands of greater intensity were observed at 1030 and560 cm^{-1}, and the appearance of a shoulder at 873 cm^{-1}. All bands are attributed to the elongation mode of PO^{3-} ions, resulting from the higher concentration of HA. The incorporation and increased concentration of HA in BC,it provided an increase in wettability, with a significant difference when comparing the control (pure BC) and theBC/HA biocomposites (p>0.05). Due to the hydrophilic character of HA and the high reactivity of Ca^{2+} ions in contact with water. Regarding the analysis of swelling and water solubility, the values found ranged from 58.65% ± 0.913 (pure BC) to 48.62% ± 10.090 (BC/0.5HA), as well as 24.54% ± 2.817 (pure BC) and 12.41% ± 3.099 (BC/0.5HA), respectively. Notably there was no significant difference between the values obtained for the biocomposites compared to the control (p>0.05). BC has a more hydrophilic character than HA and its higher proportion may have decreased these properties, even if this decrease was not sufficient to result in a statisticalsignificance (p<0.05).

Conclusions: Obtaining BC and HA biocomposites using the casting method was viable. The increase of HA concentration in the BC matrix mainly impacted the wettability of the biocomposite With increasing HA concentration, wettability increases, which is essential for cell adhesion. However, no significant differences were observed with respect to water solubility and swelling. These results indicate the possibility of performingnew in vitro and in vivo assays, and the analysis of new concentrations of BC/HA.

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Acknowledgments

The authors would like to thank the SENAI CIMATEC for supporting the development of the research. And CAPES, CNPq andFAPESB for the financial support.POROUS TITANIUM DIOXIDE-HYDROXYAPATITE COMPOSITES PREPARED BY A SOL-GEL/SUPERCRITICAL CO_2-DRYING COMBINED PROCESS.

3D BIOPRINTING AND CHARACTERIZATION OF SCAFFOLDS WITH DIFFERENT CONCENTRATIONS OF GELATIN METHACRYLATE (GelMA) FOR SKIN WOUND REPAIR

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Keywords: gelatin methacrylate; scaffolds; hydrogels; wound

Introduction and objective: In tissue engineering, the 3D bioprinting technique has been gaining prominence due to research and possible applications to aid in health, being studied to help areas like regenerative medicine,wound dressing, bone regeneration [1], and degenerative disc disease [2]. The aim of this study is to evaluate the mechanical and biological properties of hydrogels based on gelatin methacrylate (GelMA) for future application as a wound dressing carrying anti-inflammatory drugs. Hydrogels with different concentrations of GelMa will be evaluated for possible applications as a controlled drug delivery system, working as an elaboratedwound dressing.

Methodology: GelMA hydrogels were prepared at concentrations of 5, 10, and 15% w/v and dissolved in phosphate-buffered saline (PBS) together with 0.5% Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). The scaffolds (12 mm in diameter and 2 mm in height) were subjected to a UV light of 375nm for 5 minutes for the photocured reaction to occur and then were subjected to tests to evaluate their mechanical and biological properties through tests like compression, cell viability, swelling, degradation, FTIR, and rheology.

Results and discussion: Compression tests showed that the gradual increase in GelMA concentration increased resistance, and the viscosity also tended to increase the more concentrated the hydrogel was due to the rise in the concentration of GelMA in the hydrogel, which resulted in a firmer and steadier scaffold. The swelling tests showed that the higher the attention, the lower the scaffold's ability to retain water within itself, and the degradation tests showed that hydrogels with lower concentration degrade faster when compared to those withhigher concentration. This is explained because, at lower concentrations, the cross-linking is weaker between the methacrilamides, which makes the bonding between the methacrylate groups, and consequently, makes thescaffold with lower concentration absorb more water and degrade faster than the scaffold with higher concentration. The cellular viability of GelMA at each concentration was also assessed, revealing that the scaffolds were not cytotoxic and could maintain cellular life there after seven days.

Conclusions: The generated results showed the potential of GelMA regarding its use for future applications as abandage based on the increment of hydrogel concentration. Mechanical tests showed an increase in resistance,and biological showed cellular viability, which is an excellent indication for future studies with the drug incorporation and the production of a wound dress.

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POROUS TITANIUM DIOXIDE-HYDROXYAPATITE COMPOSITES PREPARED BY A SOL-GEL/SUPERCRITICAL CO_2-DRYING COMBINED PROCESS

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Keywords: TiO_2, hydroxyapatite, porous supports

Introduction and objective: Hydroxyapatite-based materials have a chemical composition like that of the mineralcomponent of bones. For this reason, these materials are the protagonists in the development of supports for osteogenesis (bone formation). The purpose of the present study is to obtain new porous supports from titaniumdioxide (TiO_2) and hydroxyapatite (HA) by a sol-gel process and supercritical-CO_2 drying. The main objective is todevelop porous scaffolds of TiO_2 with different mass proportions of HA nanoparticles and to assess the effect ofthe studied compositions on the adhesion properties of MG-63 cells.
Methodology: HA nanoparticles were synthesized by a hydrothermal method and subsequently used in a sol-gelprocess to obtain TiO_2-HA composite gels from titanium butoxide ($Ti(OBu)_4$, acetic acid, isopropanol, distilled water, and polyvinylpyrrolidone (PVP) as porogen agent. In the next step, the obtained TiO_2-HA gels of cylindricalmorphology were dried using supercritical CO_2 between 60 and 90°C at pressure from 240 to 400 bar. Finally, thedried gels were calcined at 800°C in air for 1 h. Characterization was carried out by X-ray diffraction (XRD), Ramanspectroscopy, Density/porosity measurement, Thermal analysis (TGA-TDA), Scanning electron microscopy (SEM), and *in vitro* MG-63 cell adhesion tests on dried and calcined samples.
Results and discussion: TiO_2-HA porous composites were obtained by the integration of sol-gel and supercriticaldrying processes. XRD analysis showed that between 18.6 and 29.4 % w/w of HA can be included in a TiO_2 matrixin order to avoid the phase transition from anatase to rutile. The anatase stability is increased by the increase ofHA content by means of the chemical interaction between phosphate groups in HA with hydroxyl groups in TiO_2gels, which lead to Ti-O-P bonds as confirmed by the peak at 796 cm^{-1} in the Raman spectra. Optimum conditions for drying the aerogels were at 60° C and 250 bar, TiO_2-HA composites with open porosity of 70-80% were obtained with a uniform distribution of pores of 5-20 µm in size. Furthermore, the materials obtained favor the adhesion of osteoblast-like cells and their proliferation, with the sample with the highest HA content showing the best response.
Conclusions: It was possible to obtain consolidated materials with a satisfactory response to *in vitro* adhesion tests with osteoblast-like cells. Furthermore, the developed materials presented stable crystalline phases at 800°C of anatase and hydroxyapatite, which provide specific properties to the developed biomaterial. It was alsopossible to detect the interaction of these phases, with no evidence of reactivity between them, which determines a good integration of the phases in the final structure of the composite material.

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CORE-SHELL ELECTROSPUN NANOFIBERS BASED ON SOY PROTEIN ISOLATE FOR TISSUE ENGINEERING APPLICATIONS

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Keywords: Core-shell; Nanofibers, Electrospinning; Soy Protein Isolate

Introduction and objective: The properties combination from two polymeric phases enabled by coaxial electrospinning have drawn significant attention in the tissue engineering. The core-shell structure proves instrumental in leveraging distinct polymeric phases to their fullest potential. Among the various strategic combinations, researchers have employed this structure to integrate mechanically robust polymers in the core, while utilizing more biocompatible polymers for the shell. This dual approach enhances both the mechanical resilience and biocompatibility of the resultant fibers. Electrospun soy protein isolate (SPI) is being studied to be applied in the tissue engineering field, but the obtained mats lack of optimal mechanical properties, being highly brittle. Therefore, the aim of this work is to obtain core-shell fibers using the coaxial electrospinning technique using PCL, an FDA approved polymer, and SPI as core and shell phases, respectively, and conduct morphological and physicochemical characterizations.
Methodology: The core solution was prepared by dissolving PCL (Mn=80 kDa) in glacial acetic acid, and the shell solution was prepared by dissolving SPI and oxidized sucrose (a cross-linker) in 10 mol/L acetic acid. Both solutions were electrospun using a coaxial configuration by applying optimized process parameters. A thermal posttreatment was carried out to complete the SPI cross-linking process. Morphological analysis was performed using SEM, TEM, confocal microscopy, and fluorescence microscopy. Thermal analysis was carried out using DSC and TGA; and surface chemistry study was conducted using FTIR. Lastly, the mats' wettability was analyzed through contact angle measurements.
Results and discussion: The SEM micrographs show that a fibrous structure was obtained and the average fiber diameter before and after the thermal treatment was 635 ± 128 nm and 665 ± 152 nm, respectively. A statistical analysis showed that the thermal treatment did not induce a significant change in the fiber diameter distribution. The results of TEM, confocal microscopy and fluorescence microscopy showed inconclusive results about the coaxiality of the polymeric phases. The coaxiality could not be detected through TEM micrographs because the polymer phases did not show enough density differences, therefore there was no contrast between them. Through the confocal and fluorescence microscopy, it was possible to observe that both polymeric phases were continuous throughout the fibers. However, it was not possible to determine whether the SPI phase was inside the PCL one. On the other hand, the contact angle results showed that the mats' wettability were more like the SPI fibers, which gives a hint that the fiber surface is composed by the SPI phase. Both thermal analyses yielded similar results, the mats presented an overlap of the raw materials thermal events. Lastly, the electrospun mat FTIR spectra also showed the raw material characteristic vibrational peaks.
Conclusions: The SPI-PCL coaxial mats were successfully obtained ant the characterization results showed that they have an overall great potential. The *in vitro* characterizations are ongoing to determine their aptitude inthe tissue engineering field.

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DEVELOPMENT OF MAGNETIC BONE CEMENT BASED ON CALCIUM PHOSPHATE AND IRON OXIDE NANOPARTICLES

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Keywords: magnetic bone cement, hydroxyapatite, iron oxide nanoparticles.

Introduction and objective: The purpose of this work was to contribute to the physical chemistry of innovative magnetic bone cements based on calcium phosphates (CPC) and magnetic iron oxide nanoparticles (IONPs). The focus of study was the synthesis of magnetic nanoparticles and their functionalization for protection and simultaneously for the retention of a model drug, facilitating its long-term effectiveness. Finally, it was intendedto establish the changes that may occur in the physicochemical properties, and especially in the setting reactionof the CPC by the presence of IONPs with and without functionalization.

Methodology: IONPs were synthesized by the method developed by Tsuzuki et al. [1] obtaining a product composed of magnetite/maghemite phases. This was achieved by mechanochemically processing of a mixture of $FeCl_3.6H_2O$, Fe° and NaOH during 12 h, and ulterior washing to remove saline by-products. The functionalization of synthesized IONPs with a silane (Glymo) and usnic acid (AU) was made at 300-400 bar and 40-60°C in a supercritical CO_2 equipment. Mixtures of calcium phosphates (TTCP + DCPA) containing naked and functionalized IONPs with good setting properties were developed. The influence of IONPs content in mixtures of tetracalcium phosphate (TTCP) and anhydrous calcium acid phosphate (DCPA) was studied. Characterization was carried out by X-ray diffraction (XRD), FTIR spectroscopy, thermogravimetric analysis (TGA), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and *in vitro* antibacterial assays.

Results and discussion:

The synthesis led to obtain particle agglomerates of ≈300 nm, formed by nanoparticles with a mean size of ≈9 nm and high saturation magnetization and low coercivity. The IONPs functionalized with Glymo/AU showed chemical and magnetic stability after the 30 days of incubation in simulated biological fluid (SBF) at 37°C. Functionalized IONPs exhibited a marked antibacterial activity against the strains *L. innocua* and *P. aeruginosa*. The setting reaction of the CPCs yielded hydroxyapatite (HA) crystals, process favoured by addition of 10 or 20 % IONPs (hardening times were not significantly retarded by IONPs). Promising compositions were formulated with10% of IONPs and 94 mg AU/g sample. These magnetic CPCs showed high conversion to HA, improved mechanicalproperties, hyperthermia capacity and bactericidal activity against *L. innocua*.

Conclusions: New cement formulations based on calcium phosphates and functionalized IONPs were developed.Functional properties showed the effectiveness of these multicomponent systems as theranostic materials.

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DEVELOPMENT OF A NOVEL CASHEW TREE GUMCONTAINING HYDROXYAPATITE SCAFFOLD FOR BONE REPAIR PURPOSES

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Keywords: Polysaccharides; Apatites; Bone Repair; Adipose-derived stem cells.

Introduction and objective: Cashew tree gum is a natural polysaccharide that has caught the attention of researchers because of its biocompatibility with human tissues. It is a highly available material in the Northeast region of Brazil. Hydroxyapatite and calcium phosphates-related ceramics have also been investigated because of their compatibility and chemical similarity with tissues, mainly bone, and teeth. This research aims to describe the synthesis and characterization of cashew gum/hydroxyapatite scaffold and evaluate the possible cytotoxicity in murine adipose-derived stem cells (ADSCs) cultures [1].

Methodology: Cashew gum (CG) was isolated, crushed, and washed to remove impurities. Hydroxyapatite (HAp) was synthesized via chemical precipitation in a suspension of CG. CG-HAp composite was macerated and homogenized in a gellan gum (GG) aqueous medium. Then, CG-GG-HAp scaffolds were freeze-dried. The scaffolds were characterized through scanning electron microscopy (SEM), infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermal analysis (TG and DTG), and mechanical testing. The cytotoxicity of the scaffolds was assessed on ADSCs through an MTT assay. ADSCs of the subcutaneous fat tissue of Wistar rats were collected, isolated, expanded, differentiated into three strains, and characterized immunophenotypically.**Results and discussion:** By SEM, the scaffold presented a smooth surface with aggregates of hydroxyapatite particles distributed throughout the material and morphological pores with an average diameter of 94.45 ± 50.57 µm, which are considered promising for cell growth, allowing nutrients and metabolites exchanges, once, according to [2], the size of mesenchymal cells varies from 11 to 19 µm. The DRX technique confirmed the formation of a crystalline structure for hydroxyapatite. Thermal analyses by TG/DTG revealed that the compositeis thermally stable up to 250 °C. Through FTIR, characteristic bands of all the precursor materials in the scaffold were observed. By mechanical tests, the compressive force and modulus of elasticity were like the cancellous bone. The isolated ADSCsshowed fibroblast morphology, adhesion capacity to plastic, differentiation in osteogenic, adipogenic, and chondrogenic lineages, the positive expression for the CD105 (54,2%) and CD90(56,9%) markers, and negative expression for the CD45 and CD14 markers. The MTT test showed increased cell viability at the two evaluated times, 24 and 48 h, of 111.40% and 117.62%, respectively, considering the positive control as 100% and the biomaterial showed a high level of hemocompatibility (<5%).

Conclusions: This study allowed us to describe the synthesis and the characterization of a novel cashew gum/hydroxyapatite scaffold which presented macro and microscopic characteristics for potential use as a support matrix and growth of adipose-derived stem cells. It revealed increased ADSCs cell viability and the possibility of future surgical applicability in tissue regeneration. ADSCs exhibited typically fibroblastoid morphology, plastic adhesion capacity, CD105 and CD90 immunophenotype expression, and in vitro differentiation into adipogenic, osteogenic, and chondrogenic lineages.

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TOPIC 4

Cell-biomaterial Interactions

CHARACTERIZATION OF PECVD THIN DLC FILMS ON ALUMINUM SUBSTRATE AND CELL ADHESION TEST

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Keywords: Characterization; DLC Thin Films; Cell Adhesion Test; Hydrophobic Coating.

Introduction and objective: The study of materials tribology and surface engineering is crucial to the manufacturing of Ventricular Assist Devices (VADs) including other devices and components that need to be implanted and interact with blood. Concerning this, the objective of this work was to develop a DLC film for using as biomaterial to improve tribological characteristics, characterize thin films, and evaluate its surface for cell adhesion.
Methodology: Samples of aluminum alloys 5052, 6351, and 7075 were properly polished and cleaned using ultrasonic processes. Then, amorphous silicon film was applied to samples with intention of enhancing the adhesion of DLC film which was deposited using PECVD (Plasma-Enhanced Chemical Vapor Deposition) process with precursor gases for chamber cleaning: a) Silane and Methane; and b) Argon. Subsequently, the samples underwent various characterization processes: Indentation, Nanoindentation, Goniometry, FEG-SEM (Field- Emission Scanning Electron Microscopy), and Raman spectroscopy. After these steps, the samples were subjected to a cell adhesion test.
Results and discussion: The thin film made on the samples was satisfactory and showed good adhesion to the substrate of the 3 aluminum alloys. Using a crystalline silicon test specimen, which was coated with amorphous silicon and DLC (Diamond-Like Carbon), in its cross-section using FEG-SEM (Field-Emission Scanning Electron Microscopy) with a magnification of 30,000x, it was found that the film had a total thickness of 1423.64 µm and was deposited in two hours, achieving a deposition rate of 0.71 µm/h. The DLC film is probably graphitic, due to the gas used being Silane. The contact angle measured with a goniometer was around 54.4°, indicating that the film is hydrophilic since it is below 60°. The film showed a hardness of 20 GPa in the nanoindentation test.
Conclusions: The DLC films showed good adherence to Aluminium substrate, and the deposition of the amorphous silicon film contributes to this. The Raman spectroscopy test identified that the ID/IG ratio was very close to 1, indicating that the film is likely to be graphitic. However, the films did not show good cellular adhesion, suggesting their potential application in parts of the Ventricular Assist Device where platelet and thrombus adhesion should be avoided.

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THE EFFECT OF DEGRADATION PRODUCTS FROM CHITOSAN-SILOXANE HYBRID SOLID MEMBRANES ON THE CYTOCOMPATIBILITY

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Keywords: Organic-inorganic hybrids; chitosan; degradation products; cytocompatibility

Introduction and objective: Silicon is an essential trace element for the human body, which has an effect on metabolism activity [1]. Many studies on bone regeneration by materials including silicic acid or siloxane units have been reported. Most results concluded that appropriate silicon concentration stimulated osteoblastic gene expression for their differentiation [2]. Recently, our group focuses on the effect of the chemical structure surrounding silicon on cell activity. In this study, the chitosan-based hybrid solid membranes including different silane reagents are used to investigate the cytocompatibility for osteoblastic and nerve cells.

Methodology: Chitosan was dissolved in 0.25 M acetic acid aq. to prepare 2 (w/v)% chitosan solution. Silane regents hydrolyzed with acetic acid aq. were added into the chitosan solution. The obtained precursor sols were kept under closed conditions at 60°C until gelation. After gelation, the gels were dried at 60°C. The dried membranes were neutralized with 0.2 M sodium hydroxide aq., then washed with distilled water. The extractions were prepared by soaking the dry membranes in pure water at 37°C for 7 days. The extractions were used for cell culture of osteoblastic cells (MG63) and shwannoma cells (RT4-D6P2T).

Results and discussion: The three types of silane reagents, 3-glycidoxypropyltrimethoxysilane (GPTMS), 3-glycidoxypropyldimethoxymethylsilane (GPDMS), tetraethoxysilane (TEOS), were used for chitosan-siloxane hybrids. The main peaks detected by time-of-flight mass spectrometry (TOF-MS) in the extracts from Chitosan- GPTMS, Chitosan-GPDMS, and Chitosan-TEOS hybrids were at 100-400, 100-400, and 100-600 molecular weight, respectively. It is considered that the extracts from each hybrid contain monomer-like structures of GPTMS, GPDMS, and TEOS. Also, their molecular weight is about 212, 210, and 96, respectively. The extractions did not have effects on the cell morphology for both cells. In the case of MG63 cells, extraction from Chitosan-TEOS suppressed cell proliferation at 10 µM of silicon. On the other hand, the proliferation of RT4-D6P2T was inhibited in the extracts of Chitosan-GPTMS and Chitosan-GPDMS hybrids at 5 µM of silicon. It indicates that the extracts including silicon were different molecular weights and the effects of the extracts o cytocompatibility depends on the type of cells.

Conclusions: The effects of silicon concentration and molecular weights of degradation products including silicon in the extracts were investigated. Osteoblastic cells and shwannma cells had different effects from the extracts.

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SELECTIVE CAPTURE OF PROSTATE TUMOR CELL LINES ON MULTILAYERED FILMS OF CHITOSAN AND HYALURONIC ACID

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Keywords: Prostate cancer, Cell adhesion, CD44, Multilayer films

Introduction and objective: Multilayered films of chitosan (CHI) and hyaluronic acid (HA) are commonly used in biotechnological applications due to their biocompatibility and cell adhesion properties. These films utilize the specific and natural interaction between HA and CD44, a glycoprotein overexpressed in circulating tumor cells (CTCs), to promote the adhesion of cancer cells. PC3 and DU145, both prostate cancer cell lines, exhibit high bloodstream dissemination and CTC overexpression, which provides valuable insights into cancer metastasis and detection. This study assesses CHI/HA film selectivity towards both cell lines and coculture, proposing strategies to guide cell behavior and enhance specificity in multilayer films.

Methodology: PC3 and DU145 cells were cultured in RPMI High medium supplemented with 10% fetal bovine serum and 1% streptomycin at 37°C with 5% CO2. Cell suspensions containing 6.25x104 cells (0.5 mL of PC3, DU- 145, or PC3-DU-145) were pipetted onto HA/CHI films. After incubation for 1 hour, samples were washed with DPBS buffer (pH 7.4). Adhered cells were fixed in 4% paraformaldehyde for 20 min, washed with DPBS, and stained with phalloidin (1:500) and DAPI (1:1000) for 30 min. Tumor cell adhesion was evaluated using an Axio OBSERVER.z1 ZEISS inverted optical microscope (20x objective), and cell numbers were quantified with ImageJ software. Topography images were obtained using an X Atomic Force Microscope in tapping mode. Data analysis was performed using Gwyddion open-source software.

Results and discussion: Both the PC3 and DU145 cell lines exhibited adhesion to the multilayer films. However, a statistically significant difference in cell adhesion occurred between them, PC3 cells adhered to approximately twice as many cells compared to the DU145 line. This finding corroborates previous studies that have reported the uniform distribution and high expression of CD44 in PC3 cells, while CD44 expression is low in DU145 cells [1]. In co-culture conditions, no statistically significant differences were observed, which suggests that the co- culture environment may mitigate the observed differences in adhesion between the two cell lines. Additionally, the properties of the multilayer films, such as surface roughness play a crucial role in determining cell adhesion due to the differences in cell morphology for each cell line. Underscoring the importance of understanding the characteristics of multilayer films to enhance specificity and improve their applicability.

Conclusions: The cell adhesion test with different tumor lines on HA/CHI films reveals their ability to selectively recognize the cellular behaviour through the CD44 level expressed by them. Emphasizing tumor cell-specific factors in multilayer film design provides insights to enhance DU145 detection through understanding cellular response. These findings highlight a straightforward approach to better understanding the selective capture and cell adhesion properties of HA/CHI films, which can be effectively useful in biosensing and diagnostic applications.

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EFFECTS OF pH AND SALT CONCENTRATION ON THE SURFACE PROPERTIES OF MULTI- LAYER FILMS BASED ON BIOPOLYMERS FOR MEDICAL TUMOR CELL CAPTURE DEVICES

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Keywords: multilayer polyelectrolyte; Sulfated chitosan; Hyaluronic acid; cell adhesion

Introduction and objective: Sulfated chitosan, derived from chitosan by sulphation, shows promising antithrombogenic and anticoagulant properties, relevant to biomedical applications. With another polyelectrolyte, it functionalizes the materials by depositing multilayer polyelectrolytes (PEM). Hyaluronic acid, a polyanion, has potential for tumor cell adhesion. Importantly, biopolymers and their derivatives, such as weak polyelectrolytes, provide precise control over the physical properties of PEMs by adjusting experimental conditions. These PEMs have interpenetrated layers that increase the synergy of the components, expanding their potential applications. Specifically, the study investigated the roughness, wettability, and cell adhesion properties of PEMs of these polymers under various pH and ionic strength conditions. **Methodology:** Sulfated chitosan was prepared according to Moraes et al. [1]. Glass slides, pre-coated with polyethyleneimine (pH 4.0), underwent alternating immersions in 0.1% (w/v) polyelectrolyte solutions for 10 minutes, followed by three rinsing steps. NaCl was added to the polycation as needed. The layer-by-layer technique assembled 3.5 bilayers, polyanion was the outer layer. Topography images were captured by X Atomic Force Microscope, data were analysed in Gwyddion software. Wettability analysis was made at OCA 15 contact angle meter. PC3 tumor cell adhesion was evaluated using an inverted optical microscope (Axio OBSERVER.z1 ZEISS, 20x objective), cell quantification was performed with ImageJ software. **Results and discussion:** The physicochemical properties of the developed PEM could be modulated with the pH variation and the ionic strength of the sulfated chitosan solution. As sulfated chitosan and hyaluronic acid are weak polyelectrolytes, the charge density and consequently the conformation of the chains can be modified by changes in pH and ionic strength. The constructed PEM had their surface properties more strongly affected by changing the pH from 3 to 5 than by increasing the ionic strength of the polycation solution with the addition of up to 0.1M NaCl. Topography and contact angle images indicate that increasing pH results in rougher and less hydrophilic films. Even so, all films showed adhesion capacity of PC3 cells. Previous studies with PEM formed by chitosan and hyaluronic acid showed the ability of hyaluronic acid to interact with extracellular CD44 receptors overexpressed in PC3 prostate tumor cells [2]. **Conclusions:** The scientific and technological interest in the use of natural polymers for biomedical applications stems from their inherent biocompatibility, biodegradability, versatility, and ease of controlling their properties. The surface properties of PEM made from sulfated chitosan and hyaluronic acid were modified by adjusting the pH and ionic strength of the polycation solution. This approach has been successfully employed to improve the functionality of the coatings produced, stating the potential to improve the performance of these biomaterials.

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PURE IRON FOR BIODEGRADABLE CARDIOVASCULAR STENTS: EFFECTS ON HUMAN CORONARY AORTA ENDOTHELIAL CELLS

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Keywords: biocompatibility; cell culture; surface properties; tube formation assay

Introduction and objective: As a safer alternative to permanent stents, biodegradable metals eliminate the dangerous permanence of a material in the body after it has served its purpose. Iron exhibits excellent mechanical proprieties for stents but deeper research investigating potential local toxicity is required before deeming it safe. In this work, the effects of pure iron on attachment, proliferation and angiogenic function of human coronary artery endothelial cells (HCAECs) were investigated. Pre-incubated iron was included in some assays to mimic the condition after 48 h of implantation. Surface characteristics of materials and induced hemolysis were studied to relate them with cellular assays.
Methodology: Iron (99.98 %; "Fe") and stainless steel ("SS 316L", control) discs of 6 mm diameter and 1 mm thickness were polished and sterilized by dry air. "Fe inc" samples were Fe samples incubated in complete cell culture media for 48 h. Samples surfaces were characterized by their morphology (SEM), roughness (profilometer), hydrophilicity (contact angle), and chemical composition (FTIR). HCAECs were incubated either in
a) direct, b) indirect contact with the samples, c) in contact with samples degradation products, and d) in a 3D model with embedded samples. Hemolysis was addressed by immersion of samples in 2% human red blood cells. **Results and discussion:** All samples presented smooth grooves on the surface, and Fe inc samples had a homogeneous deposit that notably increased hydrophilicity. Main results of cell assays revealed that HCAECs adhered in Fe and Fe inc samples presented round shape (unhealthy) at all time points, decreased cell number by day 5, and that degradation products of Fe affected viability by day 5. However, indirect contact with Fe and Fe inc samples did not affect neither cellular viability nor morphology during 24 h of incubation. This suggest that toxic effects of Fe might be mediated by insoluble or short-life products present during direct contact, in coincidence with other studies [1]. Angiogenic function of HCAECs was affected by Fe inc samples, denoted by the formation of a complex cellular network. This is hypothesized to be consequence of a greater incorporation of iron into the cell mediated by formation of iron-protein complexes formed during the pre-incubation treatment of samples; although more research is required. Hemolysis induced by Fe (4%) was higher than the induced by Fe inc (1%) and SS316L (1.8%), suggesting that deposits formed in Fe inc reduce the lysis of red blood cells. Hemolysis assay provides complementary information to *in vitro* cellular experiments.
Conclusions: Although iron and its alloys are proposed as biocompatible, the results of this work reflect that detrimental effects can occur on endothelial cells in contact with iron, including affectation of their function. The materials affected red blood cells in a different manner than HCAECs, highlighting the importance of evaluating toxicity in all cell types that will be in contact with materials. Further investigation about mechanisms of these negative effects is required to then modify materials properties and surface favouring biocompatibility.

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IN VITRO STUDY OF 3D PRINTED SKIN DRESSING FROM MARINE COLLAGEN

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Keywords: Marine collagen; Marine sponges; Skin wound; Wound dressing.

Introduction and objective: Skin dressings are required for the treatment of chronic wounds serving as protection from infection and stimuli of cell migration and proliferation. Moreover, collagen is the most used biomaterial for being biocompatible, bioactive, and low immunogenicity, although are mostly extracted from bovine and porcine source, but collagen from marine sponges has been emerging as an alternative. Considering the manufacturing, 3D printing has also been a promising strategy for printing skin dressings. In this sense, the aim of the present study was to evaluate the morphological characteristics and the cytotoxicity of the 3D printed skin dressing from marine collagen.
Methodology: Skin dressings were manufactured in the Octopus™ 3D printer at concentrations of 30:70, 50:50, and 10:90 (Alginate:Collagen). For SEM analysis, were recovered with gold and inserted in stubs. For the cell proliferation by alamarBlue®, 1 g of was inserted into 50 mL of culture medium (DMEM) for 24h resulting in the extract. Then, L929 and HFF-1 cells were placed into a 48-well plate in a concentration of $1x10^4$ for 24h, then the medium was changed by the extract and re-incubated. At least, alamarBlue® was inserted and incubated for 3 h. The absorbance was read in a microplate reader at 570-600 nm.
Results and discussion: SEM images demonstrated the presence of pores, micropores, and cell adhesion dispersed on a rough surface. For proliferation of L929, CG demonstrated a statistically significant difference compared to 30:70 on day 1, 50:50 and 10:90 at days 3 and 6. The 30:70 demonstrated a difference compared to 50:50 on days 1 and 3. Moreover, the 10:90 had a statistical difference compared to 30:70 on day 1 compared to 50:50 on days 3 and 6. For the HFF-1 cell line, CG demonstrated a statistically significant difference compared to 50:50 and 10:90 on day 1, while 50:50 demonstrated a difference compared to 30:70 and 10:90 had a statistical difference compared to 30:70. On days 3 and 6 were not observed a statistically significant difference.
Conclusions: 3D printed skin dressings are suitable for cell adhesion and proliferation, demonstrating that marine collagen in different concentrations are not cytotoxic for both cell lines. Moreover, the concentration of 30:70 was the best concentration for the skin dressings.

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IN VITRO GENOTOXICITY AND CYTOTOXICITY ANALYSIS OF BIOGLASS/SPONGIN SCAFFOLDS FROM MARINE SPONGES FOR BONE TISSUE ENGINEERING APPLICATIONS

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Keywords: biomaterials; biocompatibility; bioglass; spongin

Introduction and objective: Bone fractures are the most common injury in humans. In this subject, biomaterials able of stimulating and integrating into bone tissue are effective treatment for fractures. Among the biomaterials, Bioactive glasses like bioglass 45S5® (BG) are considered a gold standard due to biocompatibility and bioactivity among the biomaterials. Marine collagen extracted from marine sponges (Poriferas) (SPG) has also shown potential for bone healing. Thus, the aim of the study was to investigate the biocompatibility of scaffolds' from BG and SPG in vitro.
Methodology: The following analysis were performed: physicochemistry and morphology: SEM/FTIR; Cytotoxicity and genotoxicity: MTT/comet assay/micronucleus; mineralization potential: alizarin red staining.
Results: The results showed irregular BG particles and fibrillar-looking SPG particles in SEM. Also, for FTIR, characteristic peaks compatible with silicon oxide clusters and phosphorus oxide for BG and amine and carbon oxides for SPG. Thus, for in vitro studies, 25% and 50% extracts were not cytotoxic. Extracts were not genotoxic and both materials presented mineralization potential.
Conclusion: Cell culture findings lay emphasis on the BG and BG/SPG scaffolds potential as bone graft at lower concentrations due to the absence of cytotoxicity and genotoxicity for all cell lineages, in addition to the positive influence presented on biomineralization.

DERMATAN SULFATE/CHITOSAN POLYELECTROLYTE COMPLEXES FOR THE DELIVERY OF FLUBENDAZOLE TOWARDS TRIPLE NEGATIVE BREAST CANCER CELLS

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Keywords: Polyelectrolyte complexes, Dermatan sulfate, Flubendazole, Breast cancer, CD44 receptor.

Introduction and objective: The development of delivery systems to selectively target cancer cells has the potential to load repurposed anti-cancer drugs that otherwise could not be administrated due to systemic toxicity. Our group has previously reported the use of dermatan sulfate-chitosan polyelectrolyte complexes (DS/CT PECs) as a selective delivery platform towards cancer cells that express the CD44 receptor[1]. In this work we evaluate: (1) the capability of this nanosystem to be loaded with Flubendazole (FLU), a repurposed anthelmintic drug with poor gastrointestinal absorbance and aqueous solubility, and (2) analyze its effects in a model of triple negative breast cancer (TNBC).

Methodology: DS/CS-FLU PECs were obtained by ionotropic gelification. Their hydrodynamic diameter (Dh), size distribution (PDI) and Zeta-Potential were characterized by Dynamic Light Scattering (DLS), and by Transmission Electron Microscopy (TEM). The loading of FLU was addressed by UV spectroscopy, measuring the FLU absorbance peak at 296 nm. The TNBC cell line MDA-MB-231 was treated with FLU loaded PECs for 4 and 24hs. Cytotoxicity studies were performed by the MTT assay, and FLU loaded PEC uptake was analyzed by flow cytometry, using FLU loaded PECs synthesized with FITC marked CS.

Results and discussion: FLU loaded PECs were synthesized adding FLU to the concentrations of 200, 100 and 50µM**.** Similar loading efficiency of FLU (40%) was determined among the nanoformulations. However, 50µM FLU loaded PECs demonstrated a lower PDI and size, thus were chosen to continue with cell studies. This nanoformulation displayed a single population of 609(±72) nm, with a PDI of 0.498 (±0.16) and a Z-Potential of
+39(±4) mV. Regarding their cytotoxicity, after 4 and 24h of treatment, FLU loaded PECs show a decrease in viability similar to their equivalent free FLU (23±8% and 46±9%, respectively). Flow cytometry confirmed that MDA-MB-231 cells internalized an 85.6% (±1,8%) of FLU loaded PECs. The high level of uptake correlates to the high expression of CD44 (98,5±1% positive cells), indicating that this receptor could be involved in the internalization of FLU PECs.

Conclusions: Cell studies show that loaded PECs are able to deliver FLU to CD44-expressing cells and exert their expected cytotoxic effect. Ongoing studies are being carried out to improve PEC's size and polydispersity, however, the encapsulation of this repurposed anthelmintic with anti-cancer properties could prevent its toxic effects on healthy cells and prove an effective antitumoral strategy.

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IMMUNOLOGICAL MECHANISMS OF MOUSE MACROPHAGE CELLS TO POLY-N-ISOPROPYLACRYLAMIDE HYDROGELS

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Keywords: poly-N-isopropylacrylamide hydrogels, Immune response, biocompatibility, reactive oxygen species, immune surface receptor.

Introduction and objective: Poly-N-isopropylacrylamide (PNIPAM) hydrogels are materials that are being widely used in biomedicine as drug delivery system, cell scaffolds, and in tissue engineering due to their innate similarity to the extracellular matrix [1]. Previous studies carried out in our laboratory demonstrated that PNIPAM surfaces are biocompatible with cells of fibroblastic, renal, pulmonary and spermatic origin [2]. In order to apply them in live systems, it is necessary to study the interaction of immune system cells in contact with PNIPAM and its copolymers hydrogels due to they could influence tissue regeneration processes. The aim of our work was to evaluate the effects that PNIPAM and PNIPAM copolymerized with 3% 3-(acrylamidopropyl) trimethyl-ammonium chloride (APTAC) produce on cell viability, migration, expression of immune surface receptors and describe the biological processes related to the respiratory burst of murine macrophages exposed to different hydrogels.
Methodology: RAW 264.7 cells derived from murine macrophages were cultured for 48, 96, and 144 h in contact with PNIPAM and PNIPAM co-3% APTAC hydrogels. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole bromide and neutral red colorimetric assays and cell migration by phase contrast microscopy. The expression of immune surface receptors such as major histocompatibility complex (MCH) class II and cluster of differentiation 14 (CD14) together with the levels of reactive oxygen species (ROS), mitochondrial membrane potential ($\Delta\Psi m$) and programmed cell death by phosphatidylserine externalization were analysed by flow cytometry. Cells exposed to *Escherichia coli* homogenate were included as a positive control. Data were statistically evaluated using the Anova and Bonferroni test as a post-hoc. Differences between groups were considered significant at $p<0.05$.
Results and discussion: The results showed that PNIPAM and PNIPAM co-3% APTAC hydrogels did not alter the viability of RAW 264.7 cells at any of the evaluated times, while ROS levels, $\Delta\Psi m$ and cell death and migration process were similar to negative control cells. The surface expression of receptors for extracellular antigens CD14 and MCHII did not change in cells exposed to the different hydrogels. Although more studies are necessary to confirm the immunological mechanism activated by PNIPAM and its copolymer, the results show that these hydrogels are scaffolds that allow tissue regeneration.
Conclusions: PNIPAM and PNIPAM co-3%APTA hydrogels are biocompatible surfaces with macrophage cells due to they do not alter the main biological parameters involved in the immune response to a foreign agent.

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TOPIC 5

Drug release systems

MUCOADHESIVE DRUG DELIVERY SYSTEM WITH ENHANCED PERMEABILITY CAPACITY FOR INTRAVESICAL THERAPY

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Keywords: Bladder cancer; hydrogels; Papain; intravesical therapy

Introduction and objective: Bladder cancer (BC) represents 3% of the new diagnosis of cancer. 70% of the patients present the non-muscle-invasive type of the disease [1]. This type of BC is usually treated by the transurethral resection of the visible tumor, followed by intravesical inmuno- or chemotherapy. The instillation of chemotherapy into the bladder is not totally efficient as it faces limitations such as short residence time and permeability restrictions of the bladder mucosa [2]. Thus, the main objective of this work was to develop drug delivery formulations for intravesical chemotherapy that enhance its residence time and permeation capacity. **Methodology:** Carboxymethylcellulose (CMC) and polyvinyl alcohol (PVA) polymers were used to prepare a physical hydrogel with mucoadhesive properties. Papain, a thiol proteolytic enzyme, was added to the formulations as a permeation enhancer. The mucoadhesive capacity of the formulation was evaluated by its interaction with mucin, bioadhesion strength and retention on bladder urothelium. *Ex-vivo* drug permeation was also elucidated using Franz cells to observe the papain effect.

Results and discussion: The CMC + PVA formulations showed positive bioadhesion components, confirming their interaction with the urothelium. The significant decrease of ηb in the formulation containing enzyme suggests that papain was cleaving the glycoproteins present in the mucin. On bioadhesion strength assays, the prepared formulations required a slightly higher detachment force than the control. In terms of work of adhesion, all formulations had values higher than those recorded for the control ($p < 0.05$), confirming that there were mucoadhesive interactions between the bladder tissue and the gels. Hydrogel retention assay revealed that the formulations had higher bioadhesion capacity than the control (FITC solution), especially after the third wash (15 mL). By drug permeation analysis, it was possible to observe that the lag time decreased for CMC + PVA hydrogels with and without papain in comparison to the control. At the timepoint of 2 h, the formulation containing papain presented the highest capacity of permeation.

Conclusions: The biocompatible hydrogel containing papain as a permeability enhancer was an innovative approach for BC treatment to address the poor permeation of the tissue and short residence time. The formulation developed in this work could represent a very notable improvement in clinical practice, ensuring a longer retention time of the formulation in the bladder tissue, with a more sustained release of the drug and greater permeation capacity.

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EVALUATION OF CHITOSAN/N-ACETYL-D-GLUCOSAMINE SUTURE THREADS IN WOUND HEALING IN RAT SKIN (*Rattus Norvegicus*)

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Keywords: chitosan; healing; N-Acetyl-D-Glucosamine; suture threads.

Introduction and objective: Suture threads must exhibit susceptibility to tissue growth, be antimicrobial, non- toxic, have good compatibility and mechanical properties, as well as provide minimal tissue reaction [1], [2]. Therefore, the objective of this study was to develop biodegradable sutures made of chitosan with N-Acetyl-D- glucosamine (GlcNAc), optimize the mechanical properties under tension, gain control over the release kinetics, and evaluate the tissue healing process in rat skin.
Methodology: A 4% Chitosan solution was obtained in acetic acid, and 0.2 g of GlcNAc was added to the solution. Subsequently, the solution was spun in an injector pump into a coagulation bath of sodium hydroxide (1 mol/L). Following that, rinses with ultrapure water were performed. The threads were then tensioned at 10% and placed in an oven at a temperature of 65°C for 1 hour. The threads were characterized through mechanical tensile testing, biodegradation, release kinetics, cytotoxicity, and wound healing evaluation in the skin of 36 Wistar rats (*Rattus novergicus*).
Results and discussion: Based on the obtained results, it can be concluded that the tensile strength (N) supported by the threads falls within the standard range for surgical sutures. The biodegradation process was observed to occur within 35 days, and by day 21, despite no loss in mass, the threads already showed a 54.7% reduction in tensile strength (N). In the release assay, it was found that the drug was impregnated and released from the threads in a prolonged manner, with a maximum release of 60% occurring at 49 days. As for the release kinetics, the predominant model was zero-order, followed by Peppas-Sahlin and Hopfenberg models. In vitro cytotoxicity data for L929 cells indicated that the threads were non-toxic. Macroscopic analysis of wound healing revealed that the animals in the group treated with chitosan threads with N-Acetyl-D-Glucosamine exhibited a lower inflammatory response and a shorter time for complete wound closure. The repair process demonstrated satisfactory and significantly superior results in the N-Acetyl-D-Glucosamine group from day 14 compared to the group using the commercial Catgut thread, which showed unsatisfactory repair until the final 28-day period.
Conclusions: Thus, it can be concluded that the mechanical properties of the threads with GlcNAc, along with other evaluated characteristics such as biodegradation, cytotoxicity, and a prolonged release profile, show great potential for applications as surgical sutures. They are capable of accelerating the wound healing process, relieving pain, and minimizing infections at the surgical site due to the extended release of GlcNAc. These threads can be used in larger animals and even in human wounds.

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APPLICATION OF SUPERCRITICAL TECHNOLOGY IN THE PRODUCTION OF PLA FOAMS AND CETOPROFEN IMPREGNATION FOR CONTROLLED DRUG RELEASE

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Keywords: Polylactic acid (PLA); cetoprofen; foam production; drug impregnation

Introduction and objective: Polymeric scaffolds can be meticulously designed for various tissue engineering applications. To effectively support cell growth, they must exhibit essential characteristics such as optimal surface area, appropriate foam cell size, interconnected pores, high porosity, and more. Moreover, these scaffolds can be engineered to integrate a diverse range of components within their structure, thereby enhancing the tissue engineering process. In this study, the conditions of supercritical technologies were optimized to the formation of Polylactic acid (PLA) scaffolds and the successful impregnation of a model drug (ketoprofen) into their structure.
Methodology: The optimization of the scaffold formation process using supercritical CO_2 as an expansion agent was conducted through a two-level experimental design. The design encompassed two temperature levels (110 and 140 °C) and two pressure levels (10 and 20 MPa), with three replications at the central point. The contact time was set to 1 hour, and the expansion duration was 5 seconds. For the impregnation of ketoprofen into the foam structures, pressures of 15, 20, and 25 MPa were employed, along with a temperature of 45 °C, a contact time of 2 hours, and a decompression rate of 0.2 MPa/min. Drug release studies were conducted by exposing the impregnated foams to PBS at 37 °C.
Results and discussion: Polymeric foams were successfully produced under specific temperature and pressure conditions utilized in the experiments. Despite temperatures of 140 ºC being below the polymer's melting point (153 °C), persistent foams did not form, likely due to structural collapse during expansion. At 110 °C and 10 MPa, insufficient CO_2 incorporation into the polymer hindered foam formation. The foams formed at the central point conditions exhibited an average cell diameter of around 100 µm and 80% porosity. Conversely, at 110 °C and 20 MPa, the average diameter reduced to approximately 60 µm, with a porosity of around 90%. DSC and XRD analyses indicated changes in polymer crystallinity, even in cases without foam formation. The drug impregnation level was approximately 2%, showing a tendency to increase with higher pressure, as expected [1]. The drug release profiles displayed three distinct phases: an initial drug burst (up to 10 days), followed by a period of slow release (11th to 50th day), and concluding with rapid release due to polymer degradation (50th to 110th day).
Conclusions: Scaffolds of PLA were successfully produced using supercritical CO_2 technology, resulting in foam cells with mean diameters of 60 and 100 µm and porosities ranging from 80% to 90%. The processing of the polymer with CO_2 had an impact on its crystallinity. Ketoprofen was effectively impregnated into the formed foams without significant alteration to the foam structure. The impregnated drug exhibited a controlled release profile in PBS over a period of 110 days.

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CHITOSAN-BASED ANTIBACTERIAL FILMS FOR BIOMEDICAL APPLICATION

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Keywords: chitosan, controlled drug release, antibiotics

Introduction and objective: Implanted medical devices are associated with the risk of healthcare-associated infections (HAIs), necessitating the development of effective antibacterial strategies. Previous research has shown promising antibacterial activity of gentamicin-loaded chitosan-based films crosslinked with tannic acid and iron sulfate ($FeSO_4$) for over a month [1]. To expand on this study, we explored the use of moxifloxacin, a broad-spectrum antibiotic, as an alternative to gentamicin. The aim of this study is to compare the antibiotic release kinetics and antibacterial efficacy of the films against different bacteria strains when loaded with moxifloxacin or gentamicin.

Methodology: Chitosan films crosslinked with tannic acid and $FeSO_4$, loaded with gentamicin or moxifloxacin, were prepared as described previously [1]. Films were characterized in terms of thickness, swelling and mass loss in phosphate buffer saline (PBS), antibiotics release kinetics in PBS, quantified by HPLC-MS for gentamicin and HPLC-UV/Vis for moxifloxacin. Antibacterial activity was tested against *Escherichia coli* and *Staphylococcus aureus* by disk diffusion assay. Finally, antibacterial activity over time was assessed through an indirect assay, wherein eluates from the films were collected at various time points in Mueller Hinton broth, and then tested against the two bacteria.

Results and discussion: The comparison of gentamicin and moxifloxacin-loaded chitosan-based films displayed no significant differences in thickness, approximately 20 µm, mass loss (26-29%), and swelling (130-150%). However, notable differences were observed in the release kinetics of the antibiotics. Concentration-time measurements showed that moxifloxacin-films exhibited a more sustained release profile with a lower burst release compared to gentamicin-films. The release period extended beyond two months, and is still ongoing for both antibiotics. Interestingly, the amount of antibiotics released over time was above the minimum inhibitory concentration for gram-positive and gram-negative bacteria, *S. aureus* and *E. coli.* The antibacterial activity of the films was first corroborated by a disk diffusion assay; gentamicin-loaded films displayed halos around 18 mm for both bacteria, and moxifloxacin-loaded films halos of 21 mm and 27 mm for *E. coli* and *S. aureus*, respectively. Furthermore, the indirect antibacterial assay over time for both antibiotic-loaded films corroborate the release in PBS with continuous activity over two months against *E. coli* and *S. aureus.*

Conclusions: The comparison of gentamicin and moxifloxacin-loaded films revealed similar physicochemical properties but distinct release kinetics. The enhanced sustained release of moxifloxacin compared to gentamicin demonstrates the possibility of modulating release kinetics by changing the antibiotic structure. Both films exhibited prolonged antibacterial activity, making them promising as antimicrobial coatings for implanted medical devices. Future research will focus on these films but as coatings on polymers used as implanted medical device, such as polyethylene.

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CHITOSAN FILMS LOADED WITH DIFFERENT ANTIBIOTIC-POLYMER NANOPARTICLES

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Keywords: chitosan, alginate, nanoparticles, aminoglycosides

Introduction and objective: Chitosan and alginate are biodegradable, biocompatible and low-cost polymers that have large potential for biomedical applications, particularly in the nanomedicine field. They represent interesting options for the development of nanoparticles for drug delivery, promoting sustained release, improved bioavailability, stability, and biological performance. In this work, nanoparticles based on alginate were loaded with two medically important and broad-spectrum aminoglycosides, gentamicin and tobramycin. The obtained nanoparticles were incorporated into chitosan films developed in a previous work [1], aiming at obtaining a system with prolonged antibacterial activity.

Methodology: Alginate-gentamicin (A-G) and alginate-tobramycin (A-T) nanoparticles were prepared by the dropwise addition of an antibiotic solution (3-5 mg/mL) to an alginate solution (0.2%). The antibiotic/polymer ratio was 60% (w/w) for both systems. The nanoparticles suspensions were centrifugated at 3500 rpm to remove agglomerates. Chitosan films were prepared based on a previous publication [1]. The nanoparticles percentage regarding the chitosan mass in the films varied from 7.5 to a maximum of 30%, since at higher percentages the films become brittle. Control films containing only the antibiotic, equivalent to the amount of nanoparticles, from
4.5 to 18%, were also prepared. Films thickness, swelling and mass loss in PBS, release kinetics and antimicrobial activity against *S. aureus* and *E. coli* were evaluated.

Results and discussion: A-G and A-T nanoparticles showed diameters ranging from 80 to 120 nm and PDI of approximately 0.10-0.15. The encapsulation efficiencies varied between 95-100%. Homogeneous chitosan films loaded with the different amounts of nanoparticles were successfully obtained. Films with nanoparticles showed increased thickness (16-26µm) compared to the control films (13-19µm). For both antibiotics, films with nanoparticles swelled less (100%) than the control films (150%), regardless of the amount of antibiotic added. On the other hand, the mass loss in PBS decreased according to the amount of antibiotic added, regardless of the presence or absence of nanoparticles. For tobramycin, films containing 30% of nanoparticles had a mass loss of ~25%, while films with 15% and 7.5% had mass loss of only ~12-18%. For gentamicin, the differences were less significative: films containing 30% and 15% of nanoparticles had mass loss of 25%, and films with 7.5% had mass loss of ~20%. Release kinetics studies in PBS are ongoing, so far, all formulations showed an initial burst release in the first 2 hours. Films containing nanoparticles showed a smaller initial burst, releasing at least 20% less antibiotic than the films without NPs, resulting in a more controlled delivery. Disc diffusion analysis against *S. aureus and E. coli* confirmed the antibacterial properties of the systems.

Conclusions: Chitosan films incorporating A-G or A-T nanoparticles were successfully obtained. The presence of nanoparticles increased thickness and decreased the swelling capacity while maintaining the stability of the films in PBS. Release kinetics analysis have indicated, so far, a more controlled delivery for films containing nanoparticles. Moreover, all films had antibacterial activity against *S. aureus and E. coli*.

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STUDY OF CHAMOMILE FLOWER EXTRACT RELEASE IN POLYMERIC MATRIX OF (ENR-*g*-HA): POTENTIAL APPLICATION IN WOUND HEALING

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Keywords: Biomaterials; *Matricaria chamomilla*; Natural rubber; Hyaluronic acid

Introduction and objective: Skin tissue regeneration is a complex process with significant healthcare implications. Developing materials that facilitate the controlled release of bioactive compounds to support wound healing is of great biomedical interest. Therefore, this study aimed investigates the release profile of chamomile flower extract (CHAM), known for its bioactive properties, incorporated into a polymeric matrix composed of epoxidized natural rubber derived from trees *Hevea Brasiliensis* and grafted with hyaluronic acid (ENR-*g*-HA). These materials demonstrate promising potential for regenerative medicine applications due for their ability to promote wound healing and tissue regeneration [1].

Methodology: The synthesis of epoxidized natural rubber and grafting with hyaluronic acid (ENR-*g*-HA) was conducted as described in the literature procedure [1]. CHAM at concentrations of 1.0, 2.5, and 5.0 % was added during the final stage of the synthesis grafted material. *In vitro* release profiles were evaluated using a UV-Visible spectrophotometer model CIRRUS 80 (FEMTO) at a wavelength of 221 nm over a period of 72 hours. The experimental data was fitted to mathematical models including Korsmeyer-Peppas, Higuchi, First Order, Zero Order, and Hixson-Crowell to describe the CHAM release mechanism.

Results and discussion: The release profile of CHAM in the ENR-*g*-HA matrix followed a bi-exponential function. The burst release was observed in the first 5 hours for all samples. Within the first hour, sample containing 1.0, 2.5, and 5.0 % CHAM released approximately 0.138, 0.203, and 0.165 mg/mL of CHAM, respectively. This behaviour can be attributed to the higher exposure of CHAM on the material surface. After 5 hours, the release profile reached a plateau and remained constant until the end of the 72 hours assay. The Peppas equation or Power Law provided the best fit for the three release profiles ($R^2 > 0.9$). The polymeric matrix exhibited a diffusion-controlled release mechanism close to Fickian diffusion ($n \leq 0.5$) [2]. Therefore, the release of CHAM from the ENR-*g*-HA matrix showed an initial burst release followed by a sustained release phase. The Peppas equation described the release behaviour, indicating diffusion-controlled release. These findings contribute to the understanding of the release kinetics of CHAM from the matrix (ENR-*g*-HA) and provide valuable insights for the development of biomaterial-based delivery system for controlled release applications in the tissue engineering and wound healing.

Conclusions: The incorporation of CHAM into the ENR-*g*-HA matrix for sustained release demonstrated promising results. The grafted material enabled sustained release of CHAM at different concentrations over a determined period. Consistent with Peppas' model, the release mechanism was determined to be diffusion-based. These findings highlight the potential applications of ENR-*g*-HA as a biomaterial for targeted delivery of bioactive compounds. This study contributes to the advancement of innovative biomaterials with therapeutic potential in the field of wound healing.

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CHITOSAN FILAMENTS: EFFECTS OF COAGULATION RATE ON THE PHYSICAL-MECHANICAL AND ENCAPSULATION PROPERTIES OF KETOPROFEN

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Keywords: chitosan filaments; coagulation rate; drug delivery system; ketoprofen.

Introduction and objective: Chitosan is a polysaccharide with several biomedical applications, as it is a non-toxic, biodegradable and bioabsorbable biopolymer. Chitosan filaments are produced by wet spinning, where the filaments are obtained by extruding viscous acid solutions in an alkaline coagulation bath, where the solution precipitates in the form of filaments. The definition of the spinning/coagulation conditions are decisive for the final properties of the chitosan filaments and drug encapsulation. Thus, this work aimed to carry out the first evaluation of the effects of the coagulation rate of chitosan filaments on their physical-mechanical properties, encapsulation and release of ketoprofen.

Methodology: Solutions of 4% (w/v) chitosan in 0.244 mol/L acetic acid containing 1 mg/mL of ketoprofen were spun (45 mL/h) in coagulation baths containing 70% sodium hydroxide solution (0.1 to 2.0 mol/L) and 30% absolute ethyl alcohol, then washed and dried (33.2°C). The coagulation rate of the chitosan solution was determined as a function of the sodium hydroxide concentration. Then, the tensile strength, morphology, degree of crystallinity, load capacity, encapsulation efficiency and in vitro drug release were evaluated as a function of the coagulation rate of the systems.

Results and discussion: According to the results obtained, it was possible to observe that the tensile strength of the filaments increased with the coagulation rate, due to the structure and the degree of crystallinity obtained after the solidification of the filaments. The observed effects of NaOH concentration on the mechanical properties and the diameter of the filaments are in agreement with those observed by Albanna, *et al*. [1]. The authors observed that increasing the concentration of the coagulation agent accelerated the solidification of the chitosan filaments. On the other hand, coagulation rates allow for faster solidification of the filaments, which favors the formation of more regular cross sections, which provide a more homogeneous load distribution along the filaments, according to what was reported by Desorme, *et al*. [2]. It was observed that the carrying capacity and encapsulation efficiency of ketoprofen in the filaments exhibit a linear relationship with the rate of clotting developed during spinning, in addition, the drug release profiles were directly affected by the rate of clotting.

Conclusions: It was possible to conclude that the coagulation rate of chitosan filaments obtained by wet spinning influence properties such as morphology, tensile strength, degree of crystallinity and the loading properties of ketoprofen, which is an important factor for modulating the properties of these biomaterials. Furthermore, these chitosan filaments with ketoprofen have potential for use as absorbable surgical suture material.

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DEVELOPMENT AND EVALUATION OF NOVEL NANOSYSTEMS BASED ON MIXED POLYMERIC MICELLES TO ENHANCE PHOTODYNAMIC THERAPY

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Keywords: Photodynamic therapy (PDT); Nanosystems; Polymeric micelles (PMs); Aqueous solubility.

Introduction and objective: Photodynamic therapy (PDT) is a promising treatment for endoscopically accessible tumors. However, a major challenge is the poor solubility and self-aggregation tendency of the photosensitizer (PS) under physiological conditions, leading to decreased effectiveness [1,2]. Encapsulation of PS within nanosystems offers a potential solution to enhance solubility and prevent aggregation [1]. This study aims to investigate the most effective nanosystem for reducing PS self-aggregation and improving aqueous solubility. The physicochemical properties of different polymeric micelles (PMs) were evaluated, and their ability to encapsulate a lipophilic PS, 2,9(10),16(17),23(24)-tetrakis[(6-methylpyridin-2-yl)oxy]phthalocyaninate zinc(II) (PcZn), was assessed.

Methodology: T1107:P123 mixed polymeric micelles (PMs) were prepared by dissolving copolymers in PBS at 4°C and then equilibrating the system at 25°C. A PcZn acetone solution was added dropwise to the micellar system, followed by vigorous stirring for 48 hours at room temperature. The resulting green suspension was filtered to remove insoluble drug. Spherical PcZn-loaded polymeric micelles (PMs) in the nanosize range were confirmed by transmission electron microscopy (TEM). PMs' physicochemical properties were evaluated using dynamic light scattering (DLS), and solubility in different media was assessed through UV-Visible absorption spectra.

Results and discussion: The study focused on evaluating the effectiveness of different nanosystems for encapsulating the photosensitizer PcZn. The TEM analysis revealed the successful formation of spherical PcZn- loaded polymeric micelles (PMs) within the nanosize range of 16-33 nanometers. The comparison between the nanosystems showed that PMs containing a higher percentage (>50%) of P123 exhibited improved stability, indicated by a lower polydispersion index (PDI) ranging between 0.160 and 0.245. UV-Visible absorption spectra confirmed the successful incorporation of PcZn into all nanosystems. However, PcZn-loaded PMs exhibited slightly lower photoactivity compared to pure DMSO, suggesting some degree of aggregation. Nevertheless, the nanosystems demonstrated photostability and were more stable than those dispersed in water:DMSO (98:2) solution. Moreover, the evaluated nanosystems significantly increased the aqueous solubility of PcZn by up to 35 times, making them highly promising for encapsulating lipophilic photosensitizers like phthalocyanines.

Conclusions: Nanosystems based on mixed polymeric micelles (PM) effectively encapsulated lipophilic photosensitizer PcZn. PM with higher P123 percentage showed improved stability and reduced polydispersity index (PDI). PcZn-loaded PMs had slightly lower photoactivity but were photostable and increased PcZn's aqueous solubility. These findings suggest the potential of nanosystems for enhancing photodynamic therapy (PDT) in accessible tumor treatment. Additionally, the group is currently testing in vitro activity of the most promising nanosystems on CT26 colon carcinoma cells.

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KINETICS RELEASE OF SILVER NANOPARTICLES FROM GELATIN/ALGINATE SPONGES TO AN EFFICIENT TREATMENT OF WOUNDS

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Keywords: Biomaterials; silver release; Higuchi model; wounds management.

Introduction and objective: Polymeric sponges are a point of great interest for their potential application in the treatment of open wounds, especially due to their porous structure that provides excellent absorption of liquids. It is also crucial to take into account that these wounds are often caused by contact with contaminated sharps, which shows the importance of incorporating antimicrobial agents. Therefore, the aim of this study is to load and evaluate the release profile of silver nanoparticles within gelatin/alginate sponges.
Methodology: To evaluate the release of silver nanoparticles, two gelatin/alginate sponges were prepared: with and without a crosslinker. In order to determine the disintegration time of the sponges, they were immersed in a pH 7.4 PBS buffer, sampled at different time intervals, and the time required for complete disintegration was recorded. Afterwards, atomic absorption spectroscopy was used to quantify the amount of silver released. The data obtained were fitted to mathematical analysis to identify the best-fitting release model. In addition, the degree of swelling was determined in PBS buffer at pH 7.4 to evaluate the water absorbent capacity of the sponge.
Results and discussion: The mathematical fits of the silver release profiles revealed that both types of matrices were best fitted to the Higuchi model. In this mathematical model, two mechanisms control the release: erosion or disintegration and swelling(1). During the first three hours, it was observed in the silver release profiles that the crisscross sponge released a higher amount of silver compared to the sponge without crosslinker, in addition, the sponge with crosslinker also showed a higher percentage of swelling. However, in the case of the sponge without crosslinker, a more constant release profile was observed over the days.
Conclusions: Based on the present results, it can be concluded that the sponge with crosslinker showed superiority for treatment of wounds in comparison with the sponge without crosslinker. This is mainly due to its higher water absorption capacity and higher release of silver nanoparticles during the initial hours, which are crucial for the treatment of freshly inflicted wounds.

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NANO-IN-NANO ENTERIC PROTEIN DELIVERY SYSTEM

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Keywords: Nano-in-nano, nanogels, nanofibers, protein delivery.

Introduction and objective: Oral protein delivery holds significant promise as an effective therapeutic strategy for treating a wide range of diseases. However, effective absorption of proteins faces challenges due to biological barriers such as harsh conditions of the stomach and the low permeability of mucous membranes. To address these challenges, we present a novel nano-in-nano platform for enteric protein delivery in this work. This platform involves a coaxial arrangement comprising poly(N-vinylcaprolactam) nanogels (NGs) enclosed within nanofibers of Eudragit®L100-55 (EU). The pH-selective solubility of EU ensures NGs protection in the stomach, where fibers remain intact, and it releases them in the intestine where EU dissolves. [1]

Methodology: NGs were synthesized with *N*-vinylcaprolactam and *N,N*-methylenebisacrylamide as crosslinker in batch emulsion polymerization. Nano-in-nano platform was prepared by coaxial electrospinning with a NGs concentration of 10 mg/mL. Compositional and processing parameters were optimized, including the evaluation of EU concentrations of 150 and 200 mg/mL, different solvents and flow rates for both components and voltage. The membranes were exposed to different media, to simulate gastrointestinal tract conditions. In each environment, both the morphology and the protein release were evaluated.

Results and discussion: Thermoresponsive NGs, suitable size for biological applications, were successfully encapsulated within electrospun coaxial fibers. They have a collapsed size of 142.9 nm (PDI 0.058) and a transition temperature of 32.5 °C, and they were capable to host a model protein (ovalbumin, OVA) with a loading capacity of 0.963 mg_{OVA}/mg_{NGs} and temperature-dependent release. Degradation kinetics of the fibers show that at stomach pH, fibers lost approximately 20% of their weight, while in PBS (pH = 6.8) membranes were completely degraded after 2 h of incubation. Also, the degradation kinetic strongly depends on the EU content and on the presence of NGs. Membranes with lower EU content dissolved earlier, and for the same EU concentration, those with NGs took longer to dissolve. OVA release profiles were studied by incubating fibers for 2 h in HCl 0.1N and then incubating in PBS pH 6.8 until complete dissolution. In all cases, OVA release in HCl 0.1 N is lower than 10%, indicating that OVA is retained in the nano-in-nano system during 2 h equivalent residence time in the stomach. As expected, once the fibers were exposed to pH 6.8, they dissolved slower when containing larger EU content and NGs cargo.

Conclusions: This designed nano-in-nano system demonstrated pH-responsive behavior, enabling the controlled release of NGs with a kinetic profile suitable for therapeutic enteric treatments. It effectively protected the protein cargo in the stomach's acidic environment and facilitated its release in the duodenum at pH 6. The ability to modify the release profile simply by adjusting the concentration of EU used in the electrospinning process shows the potentiality of this nano-in-nano delivery platform.

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PRODUCTION AND CHARACTERIZATION OF NANOFIBERS TO BE USED AS CONTROLLED RELEASE TRANSDERMAL PATCHES

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Keywords: transdermal drug selivery system, nanofiber, rotary jet spinning and biodegradable polymer.

Introduction and objective: Although there are researchers who defend that the skin is an impermeable barrier, it is known today that many substances pass through the epidermis slowly and continuously, without going through the digestive tract, including de important relevance of first-pass metabolism, which suggests be an interesting route for drug administration due to its ability to reach blood vessels and systemic circulation, and may be an alternative to traditional routes of drug administration[1-2]. Thus, the objective is to produce and characterize nanofibers with drugs incorporated into them, which release different drugs in a controlled manner using the transdermal route.

Methodology: Using non-polar biodegradable polymers, polymer solutions were prepared in adequate concentrations for subsequent incorporation of lipid-lowering drugs, permeation promoters, preservatives and microencapsulating agents. Pharmaceutical grade chloroform was used as solvent for the components of the formulation. After formulating the polymeric solution with all the components present, the galenic form was taken to a rotary jet spinning equipment for the production of nanofibers to obtain the transdermal formulation and subsequently characterized by TEM, SEM, FTIR, TG, DSC techniques and the quantification of drugs using the technique diffusion by Franz cell and the drugs quantified by spectrophotometry.

Results and discussion: It was possible to produce, using the Rotary Jet Spinning technique, uniform nanofibers containing lipid-lowering drugs, permeation promoters and preservatives, which were incorporated into the nanofibers to serve as controlled-release transdermal patches. By using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), it was possible to verify the uniformity of the nanofibers without the presence of beads in their structure and/or broken and discontinuous fibers. The components of the formulation, as well as the semi-finished product, were characterized by FTIR, DSC and TG techniques, not finding a degradation product in the semi-finished product. A verification of the weight uniformity of 20 units of transdermal patches was carried out, demonstrating low weight variation between the analyzed samples. Using a Franz cell apparatus, it was possible to collect samples every 2 hours for 10 days and observe the controlled release of the drug in a uniform and continuous way, which permeated through the synthetic skin being delivered in the phosphate buffer solution of the Franz cell apparatus. The samples were analyzed using UV/VIS spectrophotometry to determine the concentration of the drug absorbed by the synthetic skin. To analyze the results, Fick's first law and first order analysis were used.

Conclusions: Is possible to conclude that the nanofibers produced by the rotary jet spinning technique prove to be an alternative for the treatment of diseases of various etiologies when using the transdermal route as the route of administration. Although further studies are required, the controlled release of drugs using Franz cells with a synthetic semipermeable membrane proved to be robust and uniform. Tests in an in vitro model using human skin and tests in a randomized human model are necessary.

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DEVELOPMENT AND CHARACTERIZATION OF ALGINATE MICROSPHERES LOADED WITH *MAYTENUS ILICIFOLIA* FOR PEPTIC ULCER TREATMENT

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Keywords: alginate; microspheres; natural polymers; peptic ulcer

Introduction and objective: Alginate is a natural polymeric biomaterial widely recognized for its diverse applications in biomedicine, particularly in tissue engineering and drug delivery, owing to its exceptional encapsulation capacity. By employing alginate microspheres, drugs can be efficiently carried and delivered within the body through a simple injection process, while various treatments for peptic ulcers exist, few have explored the combination of herbal medicine with polymeric materials. In this regard, the utilization of the *Maytenus ilicifolia* plant species has already gained substantial recognition within the country for its therapeutic properties [1-2]. This study aims to develop a microstructured device utilizing alginate spheres that encapsulate *Maytenus ilicifolia*, with the objective of evaluating its in vitro release effect. The encapsulation of the plant extract within alginate microspheres offers a novel approach for gastritis/ulcer treatment, potentially providing targeted and sustained delivery of the herbal medicine to the affected site.
Methodology: Microspheres were developed based on the process of ion gelation, where a homogeneous solution of 1% (w/v) sodium alginate was added to an aqueous solution of 2% (w/v) calcium chloride containing 5% dry extract of *Maytenus ilicifolia* using a drip system. After formation, the microspheres were removed from the solution and dried at 30°C for 48 hours. Scanning electron microscopy (SEM) and absorption spectroscopy (FT-IR) were used for characterization, and the release assay was performed using UV-VIS, with the collected samples analyzed at a wavelength of 220nm.
Results and discussion: The results demonstrated that SEM observations revealed the material as an amorphous spheroid-shaped particle with an average size of 1.083µm and areas exhibiting different phases, indicating the heterogeneity in the dispersion of *Maytenus* within the spheres. FT-IR assays confirm the presence of the herbal medicine in the alginate microspheres. Controlled release assays presented profile of 2mg/mL after 6h in saline solution reaching a 4mg/mL release pleateau from 10 up to 48h.
Conclusions: This study introduces a promising approach for gastritis and ulcer treatment by utilizing alginate microspheres as carriers for the *Maytenus ilicifolia* plant extract. The findings obtained here indicate that alginate microspheres are a promising candidate material for tissue engineering approaches. The next step would be to conduct *in vivo* assays to further validate their efficacy and potential applications.

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SELF-ASSEMBLED VESICLES OBTAINED BY ELECTROHYDRODYNAMIC TECHNIQUE USING DIFFERENT SOURCES OF PHOSPHOLIPIDS

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Keywords: electrohydrodynamic techniques, vesicles, self-assembly, TEM

Introduction and objective:
Vesicles are supramolecular structures composed of one or multiple bilayers of amphiphilic molecules that surround an aqueous compartment. Due to the characteristics and versatility of the structures, they can hold both hydrophobic and/or hydrophilic therapeutic agents. However, their production requires many steps, and they are unstable in storage. In this work, vesicles were obtained from a non-traditional method, using solid electrospun membranes as templates that form vesicles by self-assembly when dissolved in water [1]. The objective is to test different compositional parameters, raw materials' qualities and their storage stability.

Methodology:
Membranes were electrospun from a 10% m/v dissolution of polyvinylpyrrolidone (PVP-360, Sigma®) and different sources of phospholipids 5%m/v: A) high purity phosphatidylcholine 95,8% (Saporiti®), B) raw soy lecithin (Saporiti®) and C) soybean lecithin (14-29% choline basis, Sigma®) using different solvents: chloroform, ethanol, and a 1:1 mixture of both. The membranes were physicochemical characterized and stored under different conditions (temperature, vacuum) to assess their stability in time. The size and Z potential of the vesicles obtained by dissolving the electrospun membranes were evaluated by Dynamic Light Scattering (DLS). The morphology of those with the best features was evaluated using transmission electron microscopy (TEM).

Results and discussion:
Ethanol could not completely solubilize the mixture of PVP and C lecithin. Vesicles obtained from fresh membranes and those that were stored for 2 months were compared and measured by DLS, and no significant changes were observed in the hydrodynamic diameter. The membrane electrospun with phosphatidylcholine (A) using a mixed solvent was the one that best preserved its properties, as indicated by both the PDI and the diameter measurements in time. Besides, this type of vesicles showed a single peak and a negative zeta potential around - 30mV, indicating higher stability compared to the other templates composed of different phospholipid sources. The TEM inspection of vesicles (A) showed supramolecular structures of an average diameter of 97 nm. Agglomerated clusters and foreign particles were detected in systems B and C.

Conclusions:
It was possible to obtain vesicles by dissolving electrospun hybrid templates, and these solid membranes can be preserved over time. The use of high-purity phosphatidylcholine resulted in vesicles with the best properties. However, it may also be interesting to explore the application of soybean lecithin for in-situ vesicle formation, especially if there is a need to incorporate specific adjuvants agents for diverse applications. This approach could potentially yield a cost-effective products. The stability of the already formed vesicle solutions will be also assessed over time.

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BIOFUNCTIONAL POLYMERIC NANOPARTICLES PREPARED BY ELECTROSPRAYING FOR THE RELEASE OF IVERMECTIN

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Keywords: Nanoparticles; Ivermectin; Antiparasitic; Electrospraying

Introduction and objective: The development of polymeric nanoparticles is of great interest in the pharmaceutical field since they allow for the reduction of therapy costs and patient risks of toxicity [1]. They also increase efficacy, prevent premature degradation of therapeutic agents, and improve interaction with the biological environment. Electrospraying is a novel technique that enables stricter control of particle size distribution and morphology compared to traditional emulsion techniques [2]. The aims of this work are to prepare biofunctional polymeric nanoparticles using the coaxial electrospraying technique, conduct morphological and physicochemical characterization, and study the *in vitro* release profiles of ivermectin.
Methodology: By employing the coaxial electrospray technique, optimised process conditions were used to obtain biofunctional bilayer particles of PCL-ChF. The core was prepared by dissolving the synthetic polymer PCL (Mn=14 kDa) in a mixture of acetic acid and methylene chloride (AA:DCM). The surface layer or shell was prepared from solutions of the natural polymer chitosan of low molecular weight, modified with folic acid (ChF). The amount of ivermectin to be incorporated was established based on its MIC 90 - 100 µg/ml for leishmaniasis, and malaria at 5000 µg/ml. Morphological analysis was conducted using SEM and DLS images. Thermal analysis was performed using DSC. A surface chemistry study was conducted using FTIR. Encapsulated ivermectin content was determined by UV/visible spectroscopy. Additionally, in vitro ivermectin release profiles were studied.
Results and discussion: PCL particles with Ivermectin and biofunctional particles with a PCL core and ChF shell were prepared. PCLp 7:3 particles showed the smallest size (376 nm), while that PCL-I/ChFp and PCL-Ip particles presented mean diameters around 350 nm with polydispersity index values below 0.22, indicating monodisperse size distribution suitable for the intended application. The incorporation of ivermectin slightly decreased the size and modified the morphology of the particles, which may be attributed to the increased conductivity of the polymer solution. The obtained encapsulation efficiency values were above 90%, indicating PCL's good capacity to encapsulate the ivermectin agent. Thermal analysis revealed a decrease in the crystallinity of the PCL polymer in the nanoparticles after electrospray processing and an even greater decrease after the incorporation of the antiparasitic agent. FTIR results in PCL/ChF and PCL-I/ChF particles showed characteristic peaks of chitosan, indicating the presence of the biopolymer coating the surface. The ivermectin release results showed three well-defined stages. In the first stage, a delay in the release of ivermectin was observed during the first 4 hours. This may be due to the presence of chitosan on the surface, which acts as a barrier to the diffusion of the drug. In the second stage (4 to 12 h), a linear release of up to 40% of the ivermectin content was evident, following Zero-Order kinetics. Finally, 100% release was achieved at 6 days, mediated by the Korsmeyer-Peppas kinetic model (R^2 = 0.990) with a value of n = 0.39, indicating a release mechanism governed by quasi-Fickian diffusion. This indicates that the rate of penetration of the aqueous medium is much slower than the rate of relaxation of the polymer chains, allowing for values of diffusion release rates that are practically constant and independent of the ivermectin concentration gradient.
Conclusions: The PCL-I/ChF coaxial particles prepared and studied show characteristics that demonstrate their potential as a vector system for the controlled release of ivermectin.

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HYDROXYPROPYL CELLULOSE/POLY(METHACRYLIC ACID) BASED MULTI-RESPONSIVE NANOGELS FOR OVERCOMING MUCOSAL BARRIERS

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Keywords: Hydroxypropyl cellulose (HPC); responsive nanogels; mucin interaction

Introduction and objective: The mucosa is a barrier that protects the body from external agents but reduces the efficiency of medicines [1]. Several untreatable diseases involving this barrier highlight the need to develop novel carriers that promote cargo penetration through the mucosal barrier. In this regard, the higher glutathione (GSH) concentration in the mucosa and the decrease in pH in some pathological conditions can be used to trigger cargo release [2]. This work aims to obtain and characterize redox-responsive nanogels (NGs) by crosslinking polymeric chains via disulfide bonds, using HPC as seeds. The interaction of HPC-based NGs with mucin is investigated under different conditions.

Methodology: NGs were synthesized by a surfactant-free precipitation polymerization method. HPC was used as seed, and the polymerization was performed using methacrylic acid (MAA) as monomer and N,N'-methylenebisacrylamide (BIS) and/or N,N'-bis(acryloyl)cystamine (BAC) as crosslinkers. Therefore, five NGs samples were synthesized: HPC@pMAA-coBIS, HPC@pMAA-coBAC and HPC@pMAA-coBIS:BAC (BIS:BAC of 25:75,50:50, and 75:25). The interaction of HPC@pMAA NGs with mucin was studied by mucin binding efficiency, dynamic light scattering (DLS), surface Z-potential, and gel permeation chromatography (GPC) at reductive and at non-reductive conditions. Finally, as a proof of concept, release studies of curcumin loaded into the HPC-based NGs were performed by exposure at different GSH concentrations.

Results and discussion: HPC@pMAA NGs crosslinked with BIS and BAC were successfully obtained, with nanometric sizes of 200-250 nm, and low polydispersity index (<0.1). The NGs have shown a strong interaction with mucin, quantified by the mucin binding efficiency as the amount of mucin interacting with NGs after 1 h- incubation. Under normal conditions (pH 7.4 and no GSH) the mucin binding efficiency of all the samples was around 50%. Meanwhile, at higher GSH concentrations, only the NGs containing BAC enhance their interaction with mucin due to the covalent disulfide bridge formation between the NG and the free thiols of mucin. Under 10 mM GSH conditions, the fully crosslinked NG with BAC increases up to 65% of mucin binding efficiency. Through GPC studies, it is observed that NGs containing disulfide bonds bind more efficiently with mucin. Finally, the GPC and DLS studies did not show degradation when the NGs was incubated with GSH. However, the curcumin release study proved they are responsive under reductive GSH conditions. Interestingly, the decrease in pH, which mimic the mucosal pathological environment, also increased the curcumin release, highlighting the potential of these novel HPC-based NGs to overcome the mucosal barrier and enhance the cargo release in pathological conditions.

Conclusions: Novel HPC@pMAA NGs presenting responsiveness to the reductive mucosal environment were successfully synthesized. All NGs interacted with mucin, resulting in more efficient bearing-disulfide NGs under mucosal GSH. Release studies showed that bearing-disulfide NGs enhanced curcumin release in reductive mucosal conditions, and this was improved when a pathological mucosal scenario was mimicked.

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EFFECT OF POLYMER MATRIX ON ATV RELEASE BEHAVIOR

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Keywords: ATV; biomaterials; PLA and PCL, drug-delivery

Introduction and objective: Atorvastatin (ATV), a cholesterol-lowering statin, presents secondary actions such as bone anabolism. Nonetheless, high doses of this drug are required for this effect. The commercialized forms of this drug are pills for oral use and intramuscular injection, with little targeting for bone tissue. The bioavailability of ATVs can be improved by developing new controlled-release systems for this drug. Among the biodegradable polymers, polycaprolactone (PCL) and polylactic acid (PLA) have stood out and are intensively investigated. The objective of this work was to compare the release of ATV by PLA and by PCL matrices (PEREIRA et al., 2023)

Methodology: PCL- and PLA-ATV cylindrical filaments (PCL-ATVf and PLA-ATVf) were produced by hot extrusion (HME) at 60◦C and 145◦C, respectively. The filaments were characterized by scanning electron microscopy (SEM)/energy dispersive spectroscopy (EDS). Fourier-transform infrared spectroscopy (FTIR) analysis was performed to evaluate the chemical bonds, X-ray diffraction (XRD) to observe the crystallinity of the samples and Small angle X-ray scattering (SAXS). The release of the drug present in the filaments was evaluated in a phosphate buffer solution (pH 7.4) at 37 °C and rotation of 75 rpm in a UV-VIS spectrophotometer, recording the release to 8 hours.

Results and discussion: SEM analyses showed that PCL-ATVf and PLA-ATVf have a uniform and homogeneous morphology with good drug dispersion. EDS analysis detected the presence of O, C, N, F, Ca, and Na that are characteristic elements of ATV. Although it was not possible to observe ATV peaks, a PCL peaks shift in the PCL- ATVf diffractogram is probably related to the drug/matrix interaction. PLA-ATVf and PLA showed no peaks. SAXS analyses showed that PCL-ATVf presented a broad peak related to PCL and a well-defined peak characteristic of ATV. PLA and PLA-ATVf showed only a broad scattering around ATV peak position. PCL characteristic bands did not change with the presence of ATV. PLA-ATVf showed a change in stretching of the C=O group position that appears at 1750 cm^{-1} and at 1748 cm^{-1} for PLA and PLA-ATVf, respectively, indicating a possible PLA/ATV interaction. The ATV dissolution reached a maximum of 0.20% (wt) and 0.18% (wt) for PCL- ATVf and PLA–ATVf, respectively at the end of the drug release tests (8 hours). According to the FTIR analysis, this difference may be related to the morphology and the drug-matrix interaction that seems to have been more significant in PLA-ATVf.

Conclusions: This work described a well-succeeded method to obtain PCL and PLA filaments/ATV. SEM showed uniformity and EDS confirmed the presence of the drug on the filaments. XRD suggests an amorphization of ATV during the filament preparation for both PCL and PLA matrices. SAXS showed that PCL-ATVf presented PCL and ATV features. The drug release tests indicated that ATV is released faster for PLA than for PCL matrix.

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PRELIMINARY STUDY ON THE OPTIMIZATION OF CHITOSAN NANOPARTICLES PROCESSING USING THE ELECTROSPRAYING TECHNIQUE FOR EMBELIN ENCAPSULATION

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Keywords: Nanoparticles; Chitosan; Embelin; Electrospraying

Introduction and objective: The development of polymeric nanoparticles has become the subject of intense research in the field of Health by Bioengineering and Materials Engineering, as it allows the creation of highly functional systems with applications in disease diagnosis, prevention, and treatment. These nanoparticles have found significant use as biomaterials for the release of therapeutic agents. Chitosan is a natural polymer abundant in nature, holds great importance in the biomedical field due to its excellent properties of biodegradability, biocompatibility, mucoadhesion, films-forming capability, hemostatic and absorption-promoting properties. Moreover, it exhibits antimicrobial, anticholesterolemic, and antioxidant activities. The objective of this work is to optimise the processing conditions for obtaining Chitosan nanoparticles using the monoaxial electrospraying technique, as well as to encapsulate of embelin using coaxial electrospraying.

Methodology: Solutions of low molecular weight Chitosan (Merck) at 1.5% w/v were prepared using the environmentally friendly solvent, glacial Acetic Acid, in 1 M solutions [1]. For the optimization of Chitosan nanoparticle processing conditions through electrospraying, an infusion speed of 0.2 ml/h was set for the Chitosan solution, and different needle-collector distances were explored (10, 12, 15, and 17 cm). For each needle-collector distance, various potential differences were applied, ranging between 15, 17, 20, and 22 KV. For the encapsulation of embelin, a coaxial electrospraying strategy was employed, using embelin solutions in a solvent mixture of Acetic Acid: Dichloromethane (AA:DCM) with an 80:20 ratio as the core of the particles. Morphological characterization of the different explored conditions was conducted using SEM microscopy.

Results and discussion: Various processing conditions were explored to obtain Chitosan particles with different morphologies. The conditions with a shorter distance between the needle and collector (10 and 12 cm) showed higher efficiency in collecting particles, while at greater distances (15 and 17 cm), the particle collection efficiency decreased. In all cases, increasing the potential difference to 22 KV resulted in deformations in the spherical morphology of the particles. Two processing conditions yielded favourable results: the first corresponded to a needle-collector distance of 10 cm and an applied voltage of 20 KV (condition M1020), and the second corresponded to a needle-collector distance of 12 cm and an applied voltage of 17 KV (M1217). However, it was considered that the best processing conditions were obtained in the M1217 condition, as it resulted in particles with well-defined spherical morphology, with an average diameter of 250 nm and a standard deviation of 30 nm. The M1020 condition showed a smaller mean particle diameter (215 nm), but the particle size dispersion was higher (80 nm). Based on the previously optimised processing conditions, coaxial Chitosan nanoparticles with an Embelin core were processed, using an external-to-internal flow rate ratio of 2:1. The particles turned out to be spherical with average diameters of 255 nm and dispersions of 34 nm.

Conclusions: The nanoparticles obtained with spherical morphology and nanoscale size have potential as vector systems for the encapsulation and release of Embelin.

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NEW MANUFACTURING PROCESS FOR CONTROLLED DRUG DELIVERY SYSTEMS RESERVOIRS

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Keywords: Drug delivery system; Reservior; Vaccum Forming; Bioabsorbable polymer.

Introduction and objective: The development of drug delivery systems (DDS) encompasses various materials, designs, and manufacturing methods [1]. Biodegradable polymers stand out as excellent options for DDS due to their biocompatibility and elimination from the body after fulfilling their function [2]. The purpose of DDS is to reduce local dosage, increase efficiency, and minimize side effects by ensuring continuous release over time. Reservoir-based DDS show promise in this regard [2]. In this context, this study aims to develop a new manufacturing technique for reservoir-based DDS using PLDLA membranes through the vacuum forming process.

Methodology: The PLDLA membranes were manufactured using the solvent evaporation method. PLDLA PURUSORB® PLDL 7038 Carbion PURAC and chloroform grade P.A., HPLC, from the Synth brand, with a polymer concentration of 1% w/v, were used in petri dishes with dimensions of Ø60mm x 15mm. The reservoirs were formed using the vacuum forming process. The vacuum machine and its automation were designed and manufactured by the team of this study. The automation system was based on the Arduino platform. The membranes were characterized by TGA, optical microscopy, and SEM for morphological evaluation.

Results and discussion : It was possible to fabricate the PLDLA membranes using the solvent evaporation process. The manufactured membranes had a thickness ranging from 10 to 50 micra. Initially, they were opaque and rough. This occurred due to the presence of bubbles in the polymer and chloroform solution after agitation. Dissolving the polymer with gentle agitation eliminated the bubbles in the solution, and the formed membranes became uniform, without deformities, transparent, and easy to demold. A low-cost vacuum forming machine was developed, with an automated system based on the Arduino microcontroller. The material's glass transition temperature (TG) was used as the target temperature for forming the reservoirs in the material. It was possible to form reservoirs in the membranes, as evidenced by microscopy images. Some reservoirs were ruptured during the process due to excess vacuum and/or temperature.

Conclusions: The formation of membranes through the solvent evaporation process allowed the fabrication of thin enough membranes to be shaped by the vacuum forming process. The formation of reservoirs was successful, although there were ruptures in some samples. This can be avoided by improving the automated temperature control of the process. The next step of the work is to begin drug release testing.

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GOLD NANOPARTICLES AND POLYMERIC MICELLES CONTAINING DOXYCYCLINE AS A POTENTIAL ANTITUMORAL RADIOSENSITIZER NANOPLATFORM

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Keywords: polymeric micelles (PMs); gold nanoparticles (AuNPs); doxycycline (Doxi); radiotherapy.

Introduction and objective: Gold nanoparticles (AuNPs) have been widely implemented for cancer therapy since they exhibit unique quantum and surface properties. They are traditionally synthesized by chemical reduction methods involving solvent, metal salt, reducing and stabilizing agents. Block copolymers are found to have many advantages in the synthesis of AuNPs. In this work we study the use of block copolymers to produce AuNPs in a one step reaction as well as the production of hybrid AuNPs and polymeric micelles (PMs) blends; and evaluate their antitumoral activity in melanoma cells combining Doxycycline (Doxi) -a mitochondrial biogenesis inhibitor- to generate a potential antitumoral radiosensitizer nanoplatform.
Methodology: PMs based of poly(ethylene oxide)-poly(propylene oxide)-poly(ethyleneoxide) (PEO-PPO), F127 (Mw=12.6 kDa), F68 (Mw=8.4 kDa) and P85 (Mw=4.6 kDa) were studied comparing two forms of interaction with Au ions. Firstly, AuNPs were synthesized in the presence of PEO-PPO block copolymers of fixed concentrations (0.5–10% w/v): "AuNPs-PMs conjugates". In comparison, hybrid gold and PMs blends were obtained by self- assembly of amphiphilic copolymers in aqueous dispersion containing preformed AuNPs produced by the Turkevich method: "AuNPs/PMs blends". The formation of AuNPs has been characterized using UV-visible spectroscopy, DLS and TEM. Antitumoral activity of nanosystems was studied in melanoma cell lines A375, A375- G10 and Mel-J.
Results and discussion: TEM and DLS analysis revealed the successful formation of AuNPs and PMs. Particle size increased at higher percentages (% w/v) of block copolymer. AuNPs-PMs conjugates at a range of 0.5-5% w/v showed particle sizes between 100 and 150 nm, increasing drastically at 10% to 400-500 nm. Moreover, size of AuNPs/PMs blends was slightly smaller, in a range of 20-60 nm for 0.5-5% increasing up to 500 nm at 10%. Smallest hydrodynamic diameters were obtained with P85. All structures maintained stable for 20 days and block copolymers showed a tendency to prevent AuNPs aggregation in presence of Doxi. In vitro effects of free Doxi were studied using radiosensitive (A375) and radioresistant (A375-G10 and Mel-J) melanoma cell lines. All cell lines displayed a significant reduction on cell viability and metabolic activity at concentrations above 25µM of Doxi, being the radioresistant cells more affected. Also, radiosensitization was evaluated by clonogenic assays in A375-G10 cells irradiated with a gamma rays source (137Cs). At 1 Gy survival fraction was reduced from 60% on control to 28% on those pre-treated with Doxi. Cytotoxicity of AuNPs and PMs nanosystems are currently being studied.
Conclusions: This work presents a detailed study on the production of AuNPs with PEO-PPO block copolymers. A combination of characterization techniques has enabled us to understand the structure of AuNPs/PMs in aqueous dispersions. Copolymers of different size and relative lengths of the hydrophobic and hydrophilic blocks were investigated. Doxi has proven to have a cytotoxic effect on radioresistant melanoma cells. We expect that the combination of nanosystems with Doxi will lead to a novel therapeutic strategy for resistant melanoma.

SUSTAINING THE RELEASE OF TRAMETINIB FROM LAYER-BY-LAYER POLYELECTROLYTE COATED SPRAY-DRIED MICROPARTICLES

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Keywords: aliphatic polyesters; sustained release; microparticles; spray-drying;

Introduction: Tuning the bioavailability and controlling the release rate of anticancer agents is an ongoing challenge within pharmaceutical technology, towards more effective drug-carriers with enhanced therapeutic properties. Polymer-based drug delivery systems are designed to deliver a large dose of an agent to a targeted site in a controlled manner, reducing the dosage frequency and the side effects. Among the most popular biomaterials, biodegradable aliphatic polyesters attract significant interest due to their tunable properties (crystallinity, degradation rates). The present study evaluates the fabrication of a coated spray-dried microparticle system based on a poly(lactic-co-glycolic acid)/poly(ethylene adipate) copolymer to sustain the release of trametinib (GSK1120212).

Methodology: Drug-loaded microparticles were prepared *via* spray-drying method using a freshly synthesized PLGA/PEAd 75/25 polymer solution. The layer-by-layer (LbL) coating was then occurred by performing several successive immersions in chitosan (CHI) and alginate (ALG) aqueous solutions of 0.5 % w/v concentration and pH values 3.27 and 7.45, respectively. After each CHI/ALG layer, the microparticles were collected using centrifugation (10 min at 400 rpm). The produced four-layer coated microparticles were then dried overnight at RT and stored at 4^{o}C until further use. The formulations were characterized using FT-IR, XRD, DSC and SEM, and their *in vitro* dissolution rate was studied.

Results and discussion: The obtained SEM images confirmed the preparation of spherical particles in microscale, with increased diameter depended on the number of layers. The incorporation of the drug was verified by FTIR, and its presence in amorphous form was assessed by XRD and DSC studies. High drug loading capacity of 10.79
± 3.04 % was achieved, whilst the *in vitro* dissolution studies revealed a significantly enhanced dissolution ability compared to the free drug form, and a more controlled drug-release behavior as against their uncoated analogues, as well the formulations prepared with commercial PLGA. Cell and animal studies were further performed to evaluate the effect of the drug-loaded coated/uncoated microparticles on HeLa cells and experimental mice, respectively. The data suggested a limited release of the drug, however an enhanced activity in the case of the coated samples.

Conclusions: Polyelectrolyte-coating is a strategy widely used in biomedical applications, and particularly particle-based drug delivery, to reinforce certain properties, as for example effectively controlling drug-release and enhancing bioavailability. In this context, a model drug, trametinib (GSK1120212), was loaded on poly(lactic- co-glycolic acid)/poly(ethylene adipate) (PLGA/PEAd) microparticles and the ability of layer-by-layer (LbL) coating to control drug release and maintain its activity was investigated. The results confirmed the successful fabrication of nicely formed LbL-coated microparticles, and the *in vitro*, cell and *ex vivo* studies suggest a promising activity as sustained-release drug delivery vehicles.

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TOPIC 6

Functionalization of biomaterials

SYNERGISTIC EFFECT OF PEO-POLYMER HYBRID COATINGS WITH NANOPARTICLES INCORPORATION FOR IMPROVED TRIBOLOGICAL, BIOACTIVITY, AND BACTERICIDAL PROPERTIES ON TITANIUM IMPLANTS

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Keywords: Titanium; hybrid coating; bactericidal; wear

Introduction and objective: Metallic implants play a crucial role in various medical applications. Achieving rapid bone regeneration, preventing corrosion and wear, controlling metal ion release, and preventing infections are key objectives in implant development. However, osseointegration and implant durability can be affected by several factors. Furthermore, the widespread use and abuse of antibiotics have led to limited treatment efficacy due to emerging drug resistance. Therefore, the urgent need for new antimicrobial surface coatings has arisen [1]. This study focuses on the development of multifunctional coatings for metallic implants, particularly titanium alloys, to provide corrosion protection, antibacterial properties, and bioactivity.
Methodology: Oxidation of titanium alloy (Ti6Al4V) was performed using the plasma electrolytic oxidation (PEO) technique. Subsequently, a bioabsorbable polymer film containing nanoparticles (NPs) of zinc oxide was deposited on the oxidized surfaces. The coatings were characterized using scanning electron microscopy, energy- dispersive spectroscopy, X-ray diffraction, and contact angle measurements. Tribological tests were conducted, and in vitro assessments of bioactivity and antibacterial activity were made.
Results and discussion: The PEO technique successfully produced oxide layers on titanium alloy, providing a suitable surface for subsequent polymer film deposition. The presence of ZnO-NPs enhanced the antibacterial activity of the coatings. Tribological tests revealed improved wear resistance and reduced friction coefficients for the hybrid coatings compared to uncoated surfaces. Furthermore, the coatings demonstrated bioactive behavior and exhibited antimicrobial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. **Conclusions:** This study successfully developed multifunctional hybrid coatings for metallic implants, combining the PEO technique with bioabsorbable polymer films containing NPs. The coatings exhibited enhanced wear resistance, bioactivity, and antibacterial properties. These findings pave the way for the development of implant coatings with improved clinical performance and reduced infection risks, addressing the challenges associated with metallic implants.

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BACTERIAL NANOCELLULOSE AS A NEW ALTERNATIVE FOR CELL CULTURE PLATFORM

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Keywords: bacterial nanocellulose; cell culture platform; silane; plasma; biomaterials; tissue engineering

Introduction and objective: Bacterial nanocellulose (BNC) can be obtained by the excretion of the bacterium *Komagataeiobacter rhaeticus* and it consists of a three-dimensional structure, formed by cellulose nanofibers. Dried BNC has been considered a paper-like material and a suitable support for cell culture since it resembles the extracellular matrix (MEC). Due to the presence of hydroxyl (OH) groups on the BNC surface, it is possible to carry out a variety of chemical and physical modifications. In this sense, the objective of this work was the silanization and plasma modifications of the BNC surface aiming to obtain a new platform for cell growth.
Methodology: The produced paper-like BNC platforms were functionalized with -$(CH_2)_7CH_3$, -$(CH_2)_2CH_3$, -NH_2, -SH groups and by oxygen plasm at room temperature. The plasma time reactions and different amounts of chemical modifications were also considered.
Results and discussion: All BNC-based cell culture platforms have been characterized structurally by Vibrational Spectroscopy in the Infrared Region (FTIR-ATR) and X-RAY Diffraction analyses, thermally by Thermogravimetric curves (TG/DTG). The surface wettability of the BNC-based platforms has been determined by measuring the dynamic contact angle, and the morphologies aspects were analyzed by High-resolution Scanning Electron Microscopy (SEM) and by Atomic Force Microscopy (AFM). The booth applied physical (plasma) and chemical treatments (siloxanes) were effective to modify BNC in terms of surface rugosity and surface wettability (in general increasing the hydrophilic balance). Thermal properties (TG/DTG) and investigated structural analyses (XRD and FTIR) techniques confirm in terms of crystallinity and backbone structures, BCN has been not affected. The in vitro cell assays (osteoblasts and fibroblasts cells) allowed evaluation that the native and all modified BNC platforms revealed promising cell culture properties and became a target for future studies.
Conclusions: This work revealed that the two applied strategies (chemical and physical) modifications of BNC surface create a new generation of the cell culture platform by controlling surface rugosity and surface wettability (in general increasing the hydrophilic balance). The in vitro cell assays allowed evaluation that the native and modified BC platforms revealed promising cell culture properties and became a target for cruelty- free devices.

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DEVELOPMENT OF CALCIUM PHOSPHATE CEMENT COATINGS ON Ti_6Al_4V

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Keywords: calcium phosphate cements; Ti_6Al_4V; coatings; biomaterials.

Introduction and objective: Ti_6Al_4V, as other Ti alloys, is widely used for implants due to properties such as biocompatibility, Young's modulus and corrosion resistance. Many surface modifications are commonly used in these alloys to enhance osseointegration and bioactivity [1]. Calcium phosphates are a group of bioceramics that are chemically similar to bone and present bioactivity, being often applied as coatings to functionalize Ti alloys. Some calcium phosphates are also capable of forming self-setting cements (CPCs) by mixing solid and liquid phases that undergo a setting reaction [2]. This work aimed to develop a CPC coating on Ti6Al4V using a simple and cost-effective method.
Methodology: α-tricalcium phosphate (α-TCP) was synthesized by wet chemical reaction using $(NH_4)_2HPO_4$ and $Ca(NO_3)_2$. Precipitate was dried, calcined at 1350 °C and milled in ethanol. Ti_6Al_4V samples were polished with alumina paste. CPCs were prepared by manually mixing α-TCP powder and a liquid phase in different proportions. The produced cements, denominated A, B and C, were applied to the substrate by brush painting. Powder was characterized by BET and granulometry. Coatings were characterized by digital and optical microscopy and adhesion strength. Coating integrity was evaluated after immersion in simulated body fluid (SBF). pH evolution was evaluated during immersion in distilled water.
Results and discussion: The milled powder had specific surface area of 3.814 m^2/g and bimodal distribution with mean particle size of 8.49 µm. Coatings with different liquid-to-powder (L/P) ratio presented distinct aspects. Cement A had the lower L/P ratio; the excess of powder produced a coating with dense aspect and low porosity. However, the cement was difficult to apply by painting due to its high consistency and relatively low setting time, resulting in a thick and inhomogeneous coating. Cement C (higher L/P ratio) was easier to apply, but the drying time was longer due to the high amount of liquid. After drying, the coating presented a large amount of pores and some portions of the substrate remained exposed. Cement B had a balanced L/P ratio and produced a homogeneous coating that completely covered the substrate. Therefore, cement B was used to prepare the coatings for subsequent tests. Results of adhesion strength showed that the coating made with cement B meets the requirements of ISO 13779-2. Samples immersed in SBF did not show signs of significant wash out or dissolution after 21 days. pH decreased slightly from 7.8 after 2 h of immersion to 7.0 after 144 h. Average coating thickness was 83.43 µm.
Conclusions: In this work, a CPC coating was successfully developed and applied by brush painting in Ti_6Al_4V. A balanced L/P ratio produced a homogeneous coating with adequate adhesion to the substrate. Immersion in SBF did not result in wash out or fast dissolution up to 21 days. pH fluctuation was small after 144 h, indicating that the cement reaction does not lower the pH to excessively acidic values, which would be detrimental to the application of the coating on implants.

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A BIOINSPIRED STRATEGY FOR CHEMO-SELECTIVE BIO-FUNCTIONALIZATION OF POLYACRYLAMIDE HYDROGELS WITH CELL-ADHESIVE LIGANDS

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Keywords: poly(acrylamide); cell culture; biofunctionalization; luciferin chemistry

Introduction and objective: Cell-adhesive ligands mediate the attachment of cells to the extracellular matrix (ECM). Cells attach to these ligands to sense their environment, spread, migrate, and proliferate, ultimately defining their fate. Synthetic hydrogels, such as polyacrylamide (PAM), are used as ECM mimics to study cell-matrix interactions. Thereby, PAM hydrogels need to be biofunctionalized with such cell-adhesive ligands [1]. However, typically applied chemistries for biofunctionalization are not user-friendly, are expensive, and lack chemo-selectivity, impairing ligand functionality and leading to inconclusive results [2]. In this work, we present a novel bioinspired strategy to chemo-selectively functionalize PAM gels.

Methodology: A novel polymerizable comonomer (CBT-AM) featuring a cyanobenzothiazole (CBT) group was synthesized. CBT-AM was copolymerized with acrylamide and a crosslinker, via established protocols, to obtain PAM hydrogels bearing CBT groups (PAM-CBT). CBT moieties can react with biomolecules containing N-cysteine groups under physiological conditions, via luciferin click-chemistry. The novel biofunctionalization strategy was compared to the current golden standard method, known as sulfo-SANPAH (SS) chemistry. Biofunctionalization efficiency, homogeneity, and biological performance (e.g., cell attachment) were assessed for both systems.

Results and discussion: CBT-AM comonomer was synthesized from inexpensive raw materials in three steps. PAM-CBT hydrogels were prepared by adapting a standard protocol for PAM hydrogels synthesis, making this approach user friendly for regular PAM users. The final concentration of CBT groups in PAM-CBT hydrogels was finely controlled by adjusting the molar concentration of CBT-AM (0 - 4 mol%). PAM-CBT hydrogels at 1 mol% concentration showed similar swelling ratio and Young's modulus compared to that of plain PAM gels, indicating that hydrogels properties can be preserved when CBT-AM is incorporated. PAM-CBT hydrogels were easily biofunctionalized with N-Cysteine bearing biomolecules under mild conditions. The chemo-selective biofunctionalization occurred with high yields (>96%), at pH 8, room temperature, and in less than 30 minutes. The amount of immobilized bioligand was finely and reproducibly tuned. When comparing PAM-CBT hydrogels with SS-modified hydrogels (PAM-SS), PAM-CBT showed approximately 55-fold higher yield in the immobilization of ligands within a much shorter time frame (30 min *vs* overnight) and improved homogeneity. More importantly, biofunctionalized PAM-CBT gels showed faster and increased cell-adhesion of mesenchymal stem cells (MSCs), with more homogenously distributed cells and greater proliferative potential.

Conclusions: CBT-AM incorporation into PAM hydrogels enabled a highly efficient and robust bioconjugation strategy. PAM-CBT hydrogels, synthesized by regular protocols and biofunctionalized within minutes under mild conditions, proved to outperform the current golden standard in terms of biofunctionalization efficiency, homogeneity, and preserved biological function. These results highlight the importance of having efficient, reliable, and reproducible biofunctionalization strategies to generate more controlled ECM-mimicking models based on PAM hydrogels. Hence, this novel strategy is anticipated to promote more conclusive studies regarding cell-ligands interaction by enabling a better control of ligand density loading.

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ENHANCING CP-Ti GRADE 4 SURFACE WITH BIOACTIVE GLASS THROUGH PLASMA ELECTROLYTIC OXIDATION

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Keywords: biomaterials; plasma electrolytic oxidation; titanium; bioglass

Introduction and objective: Titanium alloys are widely utilized in biomaterials due to their excellent corrosion resistance, tissue compatibility, and favorable mechanical properties. This study aims to enhance the biocompatibility, osseointegration, cellular adhesion, and reduce bone resorption by incorporating bioactive glass onto the surface of CP-Ti grade 4 through plasma electrolytic oxidation. Various conditions involving electrolyte, voltage, and time variations were tested to optimize the modified surface's performance.
Methodology: CP-Ti grade 4 samples were prepared by sanding and cleaning before submitting to plasma electrolytic oxidation. The parameters varied included voltage (200 V to 400 V), time (60 s to 150 s), bioactive glass (45S5) concentration (1 g/dm³ to 10 g/dm³), and the electrolyte of calcium hypophosphite [1] at concentrations of 17 g/dm³ and 34 g/dm³ mixed with filtered water. The treated samples and as-received samples were analyzed using Scanning Electron Microscopy, Energy-Dispersive Spectroscopy, X-Ray Diffraction, and underwent tests for hardness, wettability, and bioactivity.
Results and discussion: The experimental tests demonstrated successful surface modification of commercially pure titanium grade 4 by incorporating bioactive glass, leading to improved implant interfaces and the desired parameters. Optimal results were observed with 200 V and 300 V and treatment times between 90 s and 120 s. The obtained images revealed desirable porosity on the samples, promoting osseointegration and cellular proliferation [2]. Additionally, the surface exhibited increased wettability and bioactivity, confirmed by the presence of calcium phosphates indicated by X-ray diffraction and energy-dispersive spectroscopy.
Conclusions: The study identified suitable and optimized parameters for plasma electrolytic oxidation in implants to achieve enhanced performance through surface modification and bioactive glass incorporation. By utilizing a calcium hypophosphite electrolyte, voltages between 200 V and 300 V, and treatment times between 90 s and 120 s, favorable surface modifications were achieved. These findings provide valuable insights into improving implant performance through surface engineering and the incorporation of bioactive glass.

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DEVELOPMENT OF FAST ANODIZING SURFACE TREATMENT TO OBTAIN TiO_2 NANOSTRUCTURES FOR APPLICATION IN DENTAL IMPLANTS

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Keywords: TiO_2 nanotubes; anodizing; dental implants

Introduction and objective: The clinical success of dental implants depends on osseointegration, which directly depends on the biocompatible characteristic of the material used in its manufacture. Thus, titanium is one of the most used materials for implants manufacturing. Aiming to improve osseointegration, several researchers proposed surface treatments such as anodization with fluoride containing electrolytes to produce nanotubes on titanium surfaces. However, these treatments usually take several hours, which makes their applicability to the industrial scenario unfeasible. So, the present work presents the development of a fast route surface treatment to obtain titania nanotubes in dental implants, produced in a few minutes instead hours.
Methodology: Samples were cleaned through a three-step ultrasonic bath in acetone, alcohol, and water, and then anodized at 140 V for 2 minutes using an electrolyte containing lactic acid, NH_4F, water, and ethylene glycol. The presence of an organic acid allows the use of high voltages in the anodization process without degrading the oxide layer, reducing the time required for the formation of the nanotubes. After that, it was carried out a heat treatment to improve the adhesion of the oxide layer to the substrate. Finally, samples were characterized regarding to the desirable characteristics, such as morphology, wettability, adhesion, and bioactivity.
Results and discussion: The surface treatment at 140 V for 2 minutes and subsequent heat treatment at 530 °C for 1 hour, resulted in a homogeneous nanostructured layer of TiO_2, with good adhesion to the substrate (measured by the scratch test and insertion test in a 10 PCF polyurethane block), good wettability (expressed by the contact angle of 0° against contact angle of 62.9° for non-treated titanium samples – measured through the sessile drop method and the Wilhelmy method) and enhanced bioactivity (higher cell viability, adhesion, and proliferation and similar cytotoxicity behavior when compared to non-treated titanium samples).
Conclusions: It can be concluded that it is possible to perform the synthesis of TiO_2 nanostructures on a Grade 4 Titanium substrate from fast anodizing by adding an organic acid to an electrolyte containing fluorine ions, enabling the increase of the process voltage without causing degradation of the oxide layer, since the organic acid acts as a chelator, delaying dissolution events. The result of the surface treatment with subsequent heat treatment is a homogeneous well-adhered bioactive oxide layer.

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TRIBOLOGICAL BEHAVIOR OF HYBRID COATINGS ON WE43 Mg ALLOY

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Keywords: Mg alloys; WE43; wear, hybrid coatings

Introduction and objective: Magnesium alloys have gained attention as potential bioabsorbable biomaterials, but their poor wear resistance poses a challenge to their widespread use. Hybrid coatings deposition offers a promising approach to modifying their properties [1]. In this study, the synthesis and characterization of hybrid coatings on the WE43 alloy, composed of Plasma Electrolytic Oxidation (PEO) and a bioabsorbable polymer, Polycaprolactone (PCL), are investigated.
Methodology: WE43 samples were sanded from #220 to #1200 grit, cleaned in an ultrasonic bath, and dried. Plasma Electrolytic Oxidation (PEO) was performed on the samples using an aqueous phosphate-based electrolyte. PCL polymer (Polycaprolactone) was deposited on the oxidized samples through immersion, with a concentration 10 % (w/w) in chloroform. Samples were analyzed using scanning electron microscopy (SEM), X- ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), contact angle measurements, and tribological tests in artificial saliva.
Results and discussion: SEM/EDS analysis revealed a well-adhered and homogeneous PEO layer on the Mg alloy surface, while XRD confirmed the formation of a ceramic oxide coating. The dip coating of PCL exhibited uniform coverage and thickness on the PEO layer. Wettability tests demonstrated increased hydrophobicity of the hybrid coatings compared to uncoated Mg alloy. Tribological evaluations showed improved wear resistance of the hybrid coatings, indicating their potential for reducing friction and wear in biomedical applications.
Conclusions: The synthesis and characterization of hybrid coatings on Mg alloy composed of PEO and PCL demonstrated promising results. The combination of PEO and PCL coatings improved the surface morphology, hydrophobicity, and wear resistance of the Mg alloy. These findings suggest the potential application of these hybrid coatings in biomedical fields.

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SYNTHESIS OF BACTERIAL CELLULOSE-NANOPARTICLES COMPOSITE AND EVALUATION OF BIOACTIVITY AND ANTIMICROBIAL PROPERTIES

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Keywords: Bacterial Cellulose; Silver Nanoparticles; Chronic Wounds; Komagataeibacter hansenii

Introduction and objective: Skin injuries, both acute and chronic, pose significant challenges in healthcare, necessitating effective treatments to restore tissue integrity and function [1]. This study aims to develop a composite dressing by combining bacterial cellulose membrane with bactericidal and bioactive nanoparticles (Zinc Oxide). Different routes of bacterial cellulose synthesis will be evaluated to achieve controlled production. **Methodology:** Komagataeibacter hansenii strain (ATCC 23769) was utilized with glucose, mannitol, peptone, yeast extract, citric acid, sodium phosphate, agar, and culture medium for the inoculum. Bacterial cellulose membranes grew in 125 ml Erlenmeyer flasks at 28 °C for 21 days, followed by purification. A comparative analysis of two culture media formulations was conducted, with mannitol demonstrating improved compatibility and resulting in superior membrane quality. Scanning electron microscopy (Tescan Vega 3) was employed for morphological characterization, including nanoparticle insertion. Antimicrobial, mechanical, and permeability tests were performed.
Results and discussion: The composite dressing exhibited significant potential for efficient and cost-effective treatment of chronic wounds. The integration of bacterial cellulose with Zinc Oxide nanoparticles conferred bactericidal properties to combat infection-causing microorganisms. Evaluation of different bacteria confirmed the composite's antimicrobial activity. Scanning electron microscopy analysis revealed well-defined fibers and successful incorporation of nanoparticles, enhancing the dressing's therapeutic properties. Moreover, the culture medium formulation using mannitol as the carbon source led to improved membrane characteristics, including enhanced thickness, yield, and reduced growth time. Mechanical and permeability tests demonstrated the composite dressing's strength and suitability for wound healing applications.
Conclusions: The developed composite dressing, comprising bacterial cellulose and bactericidal nanoparticles, holds great promise for the effective and economical treatment of chronic wounds. The utilization of *Komagataeibacter hansenii* as a bacterial strain for cellulose production offers a reliable method for controlled manufacturing. Further investigations are warranted to assess the long-term effects of the dressing and its viability for clinical implementation. The findings of this study contribute to the advancement of biomaterials in wound healing, addressing the pressing need for efficient therapies in public and private healthcare systems.

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FUNCTIONALIZATION OF Ti SURFACE WITH METAL PHENOLIC NETWORKS AND GREEN" SILVER NANOPARTICLES: *IN VITRO* EVALUATION OF ANTIMICROBIAL ACTIVITY AND CYTOCOMPATIBILITY

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Keywords: Metal phenolic networks, green nanotechnology, silver nanoparticles, antimicrobial activity.

Introduction and objective: In recent years, there has been a significant increase in infections associated with biofilm growth on implantable materials due to acquired bacterial resistance to conventional antimicrobial therapies. New nanotechnological strategies have been proposed to prevent these infections including antimicrobials nanoparticles and nanocoatings. In this study, we first evaluated the functionalization of Ti surface with a nanocoating of pyrogallol-based metal phenolic network (MPN-PG-Ti) and then with the deposition of silver nanoparticles (AgNPs) obtained through "green" nanotechnology (AgNPs-MPN-PG-Ti). The antimicrobial activity against *Staphylococcus aureus* (the main bacteria involved in implant infections) and the cytocompatibility in the MC3T3-E1 pre-osteoblastic cell line were evaluated.
Methodology: "Green" AgNPs were synthetized using $AgNO_3$ and gallic acid as reductant agent (pH=10). AgNPs were characterized through UV-visible spectroscopy. The average diameter of AgNPs was measured using TEM images. To form MPN-PG-Ti, Ti samples were immersed in a solution containing PG, BufferTris-HCl and $MgCl_2$ (pH=8.5) for 4h [1]. Subsequently, the MPN-PG-Ti samples were immersed in AgNPs suspension. AFM topographical and FTIR spectroscopic characterizations of AgNPs-MPN-PG-Ti were performed. The antimicrobial activity was assessed by counting the colony forming units (CFU/cm^2) after a 24h-exposure. Cytocompatibility was evaluated by acridine orange staining after 1, 2, 5 and 7d of incubation in MC3T3-E1 culture.
Results and discussion: The UV–visible spectrum of AgNPs exhibits a single sharp band at 404nm. A bimodal size distribution was observed through TEM microscopy, with the main population having an average size of 18.5nm and another population with an average size of 40nm. The formation of MPN-PG-Ti was confirmed through FTIR spectra and AFM images. The FTIR-spectrum of MPN-PG-Ti depicted peaks at 3260 cm^{-1}, 1630 cm^{-1}, 1138 cm^{-1} and 1042 cm^{-1} corresponding to –OH groups, ketonic groups, C-H and C-O vibrations, respectively [2]. The AFM images revealed that MPN-PG-Ti completely covers the Ti surface, resulting in flattened topography compared to polished Ti control. Additionally, numerous AgNPs were observed attached in isolated form on the coating. The antimicrobial effect evaluated against *S. aureus* demonstrated that the Ti control exhibited 2.85 ± 0.85 (E+06) CFU/cm^2, whereas the MPN-PG-Ti and AgNPs-MPN-PG-Ti samples reduced the CFU count by 1 and 3 orders of magnitude, respectively, compared to the Ti control. This indicates that the addition of AgNPs enhanced the antimicrobial effect of the coating. The cytocompatibility of each sample was determined by percentage of the covered area by cells. The results showed that MPN-PG-Ti and AgNPs-MPN-PG-Ti samples exhibited similar covered areas, demonstrating their similar cytocompatibility.
Conclusions: The results demonstrated that MPN-PG was formed on the Ti surface using a simple technique, and the "green" synthesized AgNPs could be effectively adhered to the MPN-PG-Ti surface, leading to a significant increase in the antimicrobial effect against *S. aureus*. Moreover, considering the cytocompatibility of MPN-PG-Ti with AgNPs for pre-osteoblastic cells, this nanotechnological strategy holds great promise as an alternative approach to combat and prevent biofilm formation and associated infection linked to Ti implants.

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ELECTROSPUN MATS BASED ON ETHYLCELLULOSE LOADED WITH FUNCTIONALIZED PORPHYRINS FOR THE INACTIVATION OF RESISTANT BACTERIA

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Keywords: nanofibrous mats; functionalized porphyrins; electrospinning; antibacterial surfaces

Introduction and objective: The increase in bacterial resistance is a pressing issue that requires immediate attention. Numerous studies have focused on the utilization of nanostructured materials in recent years. However, a significant portion of these studies still rely on antibiotics/drugs that contribute to the development of resistance in microorganisms [1-2]. The objective of this research is to fabricate electrospun mats based on ethylcellulose (EC) and incorporate two functionalized porphyrins ($TPPF_{20}$-Zn $TPPF_{20}$-Pd) for use in photodynamic inactivation (PDI) of *Escherichia coli*. This approach aims to address the issue of bacterial resistance by employing a different mechanism of action, circumventing the reliance on antibiotics.
Methodology: $TPPF_{20}$-Zn and $TPPF_{20}$-Pd were synthesized using the classical Lindsey conditions, followed by metalation with zinc and palladium, respectively. The resulting tetrapyrrolic macrocycles were then incorporated into ethylcellulose (EC) solutions in DMAc/Ethanol at concentrations of 1% and 2% w/w. Subsequently, EC/$TPPF_{20}$-Zn and EC/$TPPF_{20}$-Pd solutions were subjected to the electrospinning process to produce the electrospun mats. The characterization of the EC/$TPPF_{20}$-Zn EC/$TPPF_{20}$-Pd electrospun mats involved various analyses, including morphological, thermal, and spectroscopic studies. Microbial growth inhibition was assessed in planktonic suspension by monitoring absorbance over time in a microplate reader. Bacterial elimination was tested at the single-cell level exploiting fluorescence microscopy.
Results and discussion: The EC/$TPPF_{20}$-Zn EC/$TPPF_{20}$-Pd mats had a thickness ranging from 0.7 to 0.8 mm, with an average fiber diameter of 320-350 nm. The nanofibrous structure exhibited uniformity without the presence of beads. In terms of the hydrophobic properties of EC, thermal properties, and water uptake capacity, minimal changes were observed upon the incorporation of $TPPF_{20}$-Zn and $TPPF_{20}$-Pd. This suggests that the addition of the functionalized porphyrins did not significantly alter these characteristics of the EC matrix. However, X-ray diffraction experiments revealed changes in the broad peak of EC at 2θ = 22-25º, resulting from the incorporation of the functionalized porphyrin. Bacterial inhibition curves show that the growth rate decreases three times when the planktonic suspension is incubated with the EC/$TPPF_{20}$-Zn (2%) mat, followed by EC/$TPPF_{20}$-Zn (1%), EC/$TPPF_{20}$-Pd (2%), and EC/$TPPF_{20}$-Pd (1%) in decreasing inhibition effectiveness. Regarding *E. coli* inactivation, fluorescence microscopy experiments at the single-cell level provided insight into the capability of the material to eliminate bacteria during topical applications. Real-time experiments monitoring the fluorescence of the cell death marker propidium iodide show that the antimicrobial mat containing 2% Zn eliminates all cells in the imaging region within the first hour of irradiation.
Conclusions: The synthesis and the gram-scale production of $TPPF_{20}$-Zn and $TPPF_{20}$-Pd were successfully accomplished, and their incorporation into electrospun nanofibrous mats made of EC was achieved. The properties of the resulting materials, combined with their porous structure, make them suitable for potential applications in the inactivation of pathogenic bacteria mediated by PDI. These materials could be utilized as patches in infected wounds or integrated into biomedical devices, offering a promising alternative for combating pathogenic bacteria without relying on antibiotics.

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BIOCOMPATIBILITY ANALYSIS AND LOW-COST PRODUCTION OF PLLA

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Keywords: COLAOB2023; biomaterials; PLLA; polymers; orthopedic; tissue-engineering; biofabrication

Introduction: Biomaterials engineering aims to create a biocompatible scaffold for tissue regeneration. Several requirements, including biocompatibility, elasticity, degradation time, and cost, must be met. The high importation or synthesis costs limit its accessibility in certain countries. Market research reveals Polylactic acid (PLLA) as a commonly used but expensive polymer. It is crucial to validate the biocompatibility of new PLLA formulations and develop strategies for cost-effective production of biocompatible biopolymers to ensure wider and that is our investigation target.
Methodology: In this study, polylactic acid was synthesized through ring-opening polymerization and tested for biocompatibilityin female New Zealand rabbits. Two groups, one with PLLA (n = 6) and one without PLLA (n = 6), were examined at 6 and 9 months post-surgery. Clinical, biochemical, histological (Masson's trichrome), and histomorphometric analyses were performed to assess the injury site and confirm biocompatibility. Serological study on blood samples determined enzyme values (GOT and GPT). Statistical evaluation used non-parametric analysis of variance (Wilcoxon) with $p < 0.05$ as significant. Additionally, a cost analysis of the polymer was conducted.
Results and discussion: The Masson staining results indicate that animals implanted with PLLA have more bone tissue around the injury site. Significant differences were observed between the groups at 6 and 9 months after surgery. The Injury+PLLA group showed a 62% bone regeneration rate at 6 months, while the Lesion+PLLA group had only 30% regeneration. At 9 months, the Injury+PLLA group achieved a 66% regeneration rate, compared to 31% in the Injury No PLLA Group. Statistically, there were no significant differences in transaminase levels between thegroups. The statistical analysis found no significant differences in transaminase levels between the groups, suggesting that the end products of degradation are not harmful to the liver.. The costs per gram of imported PLLA ranged from $2.19 to $2.66, while the lab-synthesized PLLA cost $1.61, resulting in a difference of
$0.57 with TCI America™ and $1.05 with ACROS Organic™. Biocompatible biomaterial with lower production cost.
Conclusion: The findings of this research validated the viability of using ring-opening polymerization to produce PLLA derived from lactic acid. The results showcased the polymer's biocompatible and osteoregenerative properties, while also highlighting the cost-effectiveness and accessibility of the synthesis process. These results serve as an initial step towards developing a more affordable production method for PLLA.

BIOFUNCTIONALIZATION OF ZIRCONIUM WITH POTENTIAL APPLICATION IN THE BIOMEDICAL FIELD: AN *IN VITRO* STUDY

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Keywords: RGD peptide; Zirconium; Surface characterization; Biomimetic surface

Introduction and objective: Zirconium (Zr) is a promising candidate for permanent implants both in orthopedic and dental applications due to its capability of promote the growth of new bone tissue with low cytotoxicity and excellent corrosion resistance [1]. Numerous studies have focused on controlling the interaction between tissue and implanted materials by immobilizing functional biomolecules that could stimulate and interact with the extracellular matrix environment [2]. In this work, the effect of functionalization of Zr and anodized Zr (Zr60) with a bioactive peptide with arginine-glycine-aspartic acid (RGD) sequences as potential manufacturing material for osseointegrable implants to stimulate early bone integration is presented.

Methodology: The surfaces of polished Zr (non-anodized) and Zr anodized at 60 V in H_3PO_4 (Zr60) were functionalized using silanes (APTES) as coupling agents. The surface modifications were evaluated by Raman spectroscopy and X-ray photoelectron spectroscopy (XPS). The hydrophilic character of the surfaces, both non- functionalised (Zr and Zr60) and functionalized with RGD peptides (ZrRGD and Zr60RGD), was evaluated by the contact angle method. Electrochemical tests were performed in simulated body fluid solution (SBF) in order to determine the effect of functionalization treatment on the stability of Zr. Finally, the biocompatibility of the material was evaluated in an *in vitro* cell model.

Results and discussion: Surface analysis by Raman spectroscopy showed that the crystal structure of the oxide formed by anodization was predominantly monoclinic. The XPS study showed the presence of peptides on the Zr and Zr60 surfaces after functionalization, although the deposition of the organic film was not uniform. This discontinuity exposed in the Zr60RGD samples the underlying anodic zirconium oxides that have incorporated phosphates from the anodizing solution. In addition, two oxynitride peaks were recognized in the N 1s spectra on the ZrRGD surface. A slight increase in surface free energy was estimated after peptide anchoring on Zr and Zr60 samples. Anodic polarization curves and electrochemical impedance spectroscopy results indicated that both non-functionalized and functionalized with the RGD peptide surfaces present excellent corrosion resistance in SBF. The electrochemical impedance spectroscopy results showed that both types of surfaces behave as non- ideal capacitors, characteristic of passive films on valve metals. The *in vitro* cell results indicated that the MG-63 osteoblast-like cells present adhesion on both the control (Zr) and biofunctionalized surfaces. However, greater cell adhesion was observed on the surface modified with RGD peptides after 24 h and 48 h of culture.

Conclusions: The results provide evidence that it was possible to obtain, via silanization, thin films functionalized with RGD peptides on Zr, although XPS analysis indicates that the organic layer resulting from functionalization could be discontinuous. From the electrochemical results, it can be observed that the functionalization process does not produce any detriment on the surface, which maintains its favourable properties against corrosion in SBF and, in turn, increases *the in vitro* biocompatibility of the material at short times.

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COMPARATIVE ANALYSIS OF THE BIOCOMPATIBILITY IN POLY LACTIC ACID (PLA) SAMPLES AFTER PLASMA SURFACE

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Keywords: Biomaterials; PLA; kINPen; biocompatibility.

Introduction and objective: The global market for biomaterials is growing gradually, with revenues expected to reach 47.5 billion dollars by 2025, and medicine is driving this growth due to the increase in demand for implants that use biomaterials as raw material [1]. Among them, PLA is a polymer from renewable sources that has good mechanical properties, low environmental impact and it is absorbed by the body after its degradation. However, the inherent hydrophobicity of this biomaterial limits cell adhesion on its surface [2]. The present study aims to evaluate the improvement in PLA biocompatibility after kINPen® IND plasma treatment for future application in medical implants.
Methodology: The PLA samples were produced with the Creality ENDER 6 3D printer, with 13mm diameter and 3mm thickness. After the sterilization process, the surfaces of half of the specimens were treated with the kINPen® IND cold plasma jet for an exposure period of 90s and subjected to the wettability test using the Ramé- Hart 100-00 Goniometer. After a second sterilization process, the cell adhesion test was performed using the MEF L929 (Mouse Embryonic Fibroblast L929) cell. The samples were incubated for 24 hours at 37°C and 5% CO2. Cell adhesion was checked with Scanning Electron Microscope (SEM).
Results and discussion: The results analysed through the images obtained by SEM, demonstrated great effectiveness in the aspect of improvement in cell adhesion on the surface of PLA. An increase in fibroblasts adhered to the surface of the sample was observed, compared to the one that was not subjected to surface treatment with the plasma jet, proving the effectiveness of surface treatment using kINPen®. Making a parallel with the wettability test, it was noted that the results are in line with recent studies, which have shown that, in some types of cells, the more hydrophilic the surface, the greater the likelihood of greater cell adhesion. It is important to note that wettability is an important factor for cell adhesion, but not the only one. Other factors, such as surface roughness and chemical composition, can also influence this aspect.
Conclusions: It was observed in the PLA samples a significant improvement in cell adhesion and a contact angle with a more hydrophilic characteristic than that presented in the sample without surface treatment. According to the results obtained, it is possible to preliminarily conclude that the use of kINPen® plasma for surface treatments in samples made via FDM can present satisfactory results in terms of improvement in hydrophilicity and cell adhesion.

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DETERMINATION OF RADIORESISTANCE OF CONTAMINANT BACTERIA IN NILE TILAPIA SKIN USED AS A DRESSING

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Keywords: Biomaterials; Radioresistance; Nile Tilapia skin; Burns

Introduction and objective: The use of Nile Tilapia (*Oreochromis niloticus)* skin in regenerative medicine, in the form of biological dressing for burns, is satisfactory because this biomaterial presents good adherence to wounds and characteristics suitable for the healing process, such as the high concentration of collagen type I. The treatment with the skin provides protection and decreases the number of dressing changes, reducing the patient's pain. Before use, Nile Tilapia skins need to be decontaminated and sterilized ensuring safety, since they can be contaminated by bacteria during handling. Each bacterium has a radioresistance value, which inactivates 90% of the population of that species.
Methodology: Some human contaminations at the time of handling this material: *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Pseudomonas putida*. In this work we irradiated the isolated bacterial populations from the Nile Tilapia skin with increasing doses of ionizing radiation in culture medium. Serial dilutions were performed from the total population, after gamma-ray irradiation, to enable the identification of the remaining bacterial concentration for each dose of irradiation tested.
Results and discussion: In the literature it is possible to find radioresistance to these bacteria contained in other substrates. However, it is necessary to standardize the methodology to determine the radioresistance of microbial species in the skin of Nile Tilapia, to ensure patient safety during the use of the dressing. For *Enterococcus faecalis* we obtained a value of 0.7 kGy, very similar to that described in the literature. For *Pseudomonas aeruginosa* the value found was 0.5 kGy and for *Pseudomonas putida* it was 0.2 kGy. Thus, the standardization of the methodology is in accordance with normality, also allowing the determination of radioresistance for some bacteria not described in the literature.
Conclusions: It was possible to estimate the values of the doses necessary for the reduction of 90% of the bacterial population. The radiation doses were standardized to sterilize with a safety level of 10-6, but without reaching values harmful to the histological and molecular structure of the biomaterial (above 25 kGy), so that the biological dressing is used in patients safely and doesn't lose its physicochemical characteristics.

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CHITOSAN-GELATIN/BIOACTIVE GENTAMICIN DOPED GLASS PARTICLES SYSTEM FOR COATING TITANIUM ALLOY IMPLANTS

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Keywords: permanent implants; mesoporous particles; antibacterial effect; electrophoretic deposition.

Introduction and objective: Titanium based implants have long been studied and used for applications in bone tissue regeneration, thanks to their outstanding mechanical properties and appropriate biocompatibility. However, many implants struggle with osseointegration and attachment, and can be vulnerable to the development of infections [1]. The aim of this work is to generate and characterize repetitive, adherent, hemocompatible and antibacterial chitosan-gelatin and bioactive glass particles coatings loaded with gentamicin by electrophoretic deposition, for implantation purposes.
Methodology: Mesoporous glass particles (MBG) were produced using a sol-gel modified Stöber process and loaded with an ethanol solution with 50 µg/mL of gentamicin sulphate (Ge). Disc-shaped Ti-6Al-4V samples were polished and coated with a solution of 0.5 g/L chitosan, 1 g/L gelatin and 2 g/L MBG-Ge nanoparticles by lectrophoretic deposition (EPD)[2]. “Regular” or “enhanced” stirring and sonication (30 min stir-20 min sonic or 40 min stir - 40 min sonication, respectively) of the solution were assayed before EPD. After EPD, an algorithm based in Digital Image Processing was applied to quantify the cover area. The optimum coatings were characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), adhesion tape, antibacterial and hemolysis assays.
Results and discussion: Electrophoretic composite coatings of chitosan/gelatin with MBG-Ge particles on Ti6Al4V samples could be successfully done, generating hydrophilic, homogeneous and well adhered films. The MBG-Ge particles act as second phase and are well dispersed on the matrix. With an enhanced stirring and sonication method of the solution, it was possible to obtain a better distribution and cover area of particles in the surface (regular: 45%, enhanced: 76%). The antibacterial effect of the complete coating, attributed to the release and dissolution of the MBG-Ge particles, was evidenced for *E.scherichiacoli* and *Staphylococcus aureus*after 24h of contact. The hemolysis rate should be less than 5% for being considered hemocompatible. Hemolysis rate of Ti6Al4V, Ti6Al4V coated without Ge particles and Ti6Al4V coated with MBG-Ge were 5.1 ± 1.7, 6.5 ± 0.5 and 3.1 ± 0.2%, respectively. These results indicate that the MBG-Ge coating improves the hemocompatibility of the Ti alloy.
Conclusions: In this work, an electrophoretic approach to develop an antibacterial coating on titanium substrate was investigated, with the use of chitosan, gelatin, mesoporous bioactive glass particles and gentamicin as components. A good adhesion, particle distribution, antibacterial and hemocompatibility properties were obtained.

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BIOCERAMIC COATING ON METAL SUBSTRATE FOR BIOMEDICAL APPLICATIONS

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Keywords: fluorapatite; coatings; titanium; implants.

Introduction and objective: Fluorapatite has shown great potential in biomedical applications, due to the presence of fluorine in its structure it´s almost insoluble, has high biocompatibility, excellent bioactivity, and antibacterial behavior. All these properties make it a great choice for use as coating on metal implants, component of toothpaste and for regeneration of bone defects, for example**.** Furthermore, when compared to hydroxyapatite, the fluorapatite present better physicochemical properties such as crystallinity, osteointegration and cell proliferation. In view of this, the present work aims to obtain a titanium surface coated with fluorapatite and investigate the possibility of future use in dental implants.
Methodology: For this work, the methodology was carried in five steps, first the fluorapatite was synthesized from calcium fluoride and hydroxyapatite, which was obtained by wet synthesizing. In the second step, the metallic substrate was prepared. Subsequently the dispersion of fluorapatite in pine oil was performed, obtaining a paint for coating. And for the fourth step, the coating process on the substrate was carried out with a brush and thermally treated in a oven. Finally, the product obtained was characterized for its chemical and morphological behavior.
Results and discussion: To prepare the substrates, the anodization process was carried out, making it possible to obtain a more hydrophilic surface compared to the titanium surface that was only polished. This surface modification is an important factor when it comes to cell adhesion and protein adsorption in biological systems. X-ray diffraction (XRD) and Fourier transformed infrared spectroscopy (FTIR) results showed that there was a replacement of OH^- by F^-, without formation of other phosphate phases. With the introduction of F^- was possible to observe in FTIR analysis that the band at 630 cm^{-1} shifts to higher wavenumbers, as already proven by other authors. The laser granulometry analysis showed coarse grains of fluorapatite, which is presented by other studies that the size is influenced by the sintering temperature. After the coating was carried out, the phase was maintained, and a homogeneous coating was presented.
Conclusions: The results show that the methodology used for the elaboration of the research was effective and showed promised results. However, some tests are still necessary regarding the capacity of release of F- ions, since there is a great influence on cell attachment and proliferation.

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CAPES

FUNCTIONALIZATION OF NYLON THREAD WITH CHLORHEXIDINE AS A TOPICAL PROPHYLACTIC ALTERNATIVE FOR SURGICAL SITE COMPLICATIONS

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Keywords: Biomaterials; Suture Thread; Plasma functionalization; antibacterial

Introduction and objective: Surgical suturing is crucial for achieving favourable aesthetic and anatomical outcomes while minimizing postoperative complications. Nylon thread and chlorhexidine are commonly employed for wound closure and infection prevention, respectively. Combining these components presents a notable therapeutic advancement for surgical sites at risk of infection. However, surface modification of the surgical suture is necessary to facilitate drug binding and prevent its removal during suturing. This study evaluated the effectiveness of using chlorhexidine functionalized nylon thread, using plasma, in bacterial cultures. The aim is to reduce surgical complications [1] and the need for systemic antibiotics.

Methodology: The suture thread was chemically functionalized with chlorhexidine through surface exposure to oxygen plasma [2], subsequently immersed in a chlorhexidine solution for incorporation. Antimicrobial potential was assessed against three bacterial species: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Furthermore, the suture thread underwent characterization utilizing analytical techniques including Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and mechanical assays.

Results and discussion: The effective chemical functionalization of chlorhexidine in the suture thread was confirmed through the evidence found in the FTIR and SEM analyses. The microbiological assay obtained from agar diffusion testing after 24 hours of incubation demonstrated that the chlorhexidine present in the suture thread was capable of forming inhibition zones against *S. aureus* and *E. coli*, with inhibition zone diameters ≥ 12 mm. Chlorhexidine acts by destroying bacterial cell membranes, contributing to its bactericidal effect. However, no inhibitory effects were observed against *P. aeruginosa*, likely due to its high bacterial resistance. Mechanical testing demonstrated that the treated yarn exhibited a slight increase in tension and tensile strength compared to the untreated surgical suture. Moreover, its high Young's modulus led to reduced strain (%) and enhanced mechanical strength.

Conclusions: The chemical functionalization of chlorhexidine in the surgical suture thread proved effective, as confirmed by FTIR and SEM analyses. The microbiological assay demonstrated the potential of the chlorhexidine- treated thread to create inhibition zones against *S. aureus* and *E. coli*, indicating its bactericidal properties. Furthermore, the slight increase in mechanical strength observed in the treated surgical suture may contribute to enhanced material stiffness.

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CAPES-Brazil and PIPEQ AUXP PUCSP.

SURFACE MODIFICATION OF Fe35Mn ALLOY USING ANODIC OXIDATION

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Keywords: biodegradable metals, Fe35Mn alloy, anodic oxidation.

Introduction and objective: Biodegradable metals, such as iron-based alloys, are a new alternative for implantable materials whose functions in the body are temporary, as they have high mechanical strength and high ductility, but have a very slow degradation rate [1]. To improve the degradation rate and its mechanical properties, it is necessary to consider three factors: the addition of alloying elements to iron, such as manganese, which provides an increase in mechanical strength and degradation rate, processing and surface modification, such as growth of iron oxide nanostructures [2].
Methodology: To obtain nanotubes on the surface of the Fe35Mn alloy by anodic oxidation initially, the parameters defined for pure iron (control group) were adopted: electrolyte based on ethylene glycol, NH4F and water, voltage of 50V, agitation of 100 rpm, time of 30 min and calcination in air. Subsequently, the water content in the electrolyte composition, voltage and time were varied. The morphology of the structures formed after anodic oxidation was analyzed by scanning electron microscopy, the analysis of the crystalline phases formed was performed by X-ray diffraction and the wettability of the surfaces was evaluated by the contact angle.
Results and discussion: The anodization parameters of pure iron do not apply to the Fe-35Mn alloy, but even varying the water content of the electrolyte, the potential and the time it was not possible to obtain the formation of a nanotubular structure. A predominance of austenite phase was obtained for the Fe35Mn alloy samples and although it did not form nanotubes for any of the conditions adopted, the surfaces of the samples presented a hydrophilic character.
Conclusions: There are currently no studies in the literature on anodization in Fe-Mn alloys, making this study unprecedented and a great challenge. However, the results obtained are promising for the functionalization of iron-based biomaterials.

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TOPIC 7

Key enabling technologies (including lab-on-a-chip, artificial intelligence)

MICROFLUIDIC CIRCUIT APPLIED TO THE CONCENTRATION OF 18F FOR THE PRODUCTION OF RADIOPHARMACEUTICALS

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Keywords: Microfluidics; Radiopharmaceutical; Fluorine-18; Positron emission tomography

Introduction: Microfluidics is becoming a promising technology for synthesizing [^{18}F]-labeled radiopharmaceuticals, reducing costs, reagents, and increasing activity [1]. Conversely, current commercial production of such radiopharmaceuticals for clinical diagnosis by positron emission tomography (PET) imaging requires dedicated and expensive equipment, only available in specialized facilities to produce only one type of ^{18}F radiopharmaceutical [2]. So, as the demand for PET increases, the use of microfluidics becomes essential for this commercial production, and, in this sense, this work presents the results of a developed "micro-cartridge" microfluidic chip applied to the ^{18}F retention and elution process that can improve all the production aspects.
Methodology: The micro-cartridge was machined in borosilicate optical glass – BK7 using the ultrashort pulse laser ablation technique. After micromachining, the micro-cartridge is filled with the same resin used in the conventional anionic synthesis cartridge (Waters Accel Plus QMA Light cartridge). Both are later submitted to comparative performance tests to evaluate the radiochemical efficiency in the ^{18}F retention and elution phase between them.
Results and discussion: Four comparative tests were performed for both phases (first stage of synthesis of radiopharmaceuticals labeled with ^{18}F), with activities (55.5 ± 11.1 Mbq and 9.2 ± 0.4 Gbq; n = 2). The results showed that the micro-cartridge is equivalent to the conventional cartridge (QMA Plus Light) in the retention phase, presenting a radiochemical efficiency of 99.3% ± 0.7 vs 99.6% ± 0.3, respectively. However, in the ^{18}F elution phase, the micro-cartridge showed a radiochemical efficiency of 93% ± 0.2, and the conventional cartridge had a maximum of 77.4% ± 15.5, showing the great advantage of the micro-cartridge. The hypothesis that supports the superiority of the results of micro-cartridge efficiencies in the elution phase is the high surface-volume ratio, which leads to the prevalence of surface phenomena such as mass transfers and faster reaction syntheses, which occur in microfluidic systems. Although the microfluidic systems studied for radiopharmaceuticals have existed for almost 20 years, the use of the ultrashort pulse laser technique and the type of material used in the micro-cartridge development are not commonly reported.
Conclusions: Integrating an anion exchange micro-cartridge on a chip with the ultrashort pulse laser ablation technique opens the door to smaller, and more efficient radiopharmacy chips for producing ^{18}F radiopharmaceuticals. The first unprecedented experimental results in Brazil demonstrate that the initial stages of production of ready-to-use doses for humans (pre-concentration of fluorine) can be carried out with greater efficiency in the elution parameters of ^{18}F compared to synthesis with a conventional cartridge.

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ALGORITHM FOR RECONSTRUCTION OF TIME SIGNALS AND ARTIFICIAL NEURAL NETWORKS FOR TAXONOMY OF THROMBI IN VENTRICULAR ASSIST DEVICES

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Keywords: Ventricular assist device; thrombus; artificial neural network; predictive maintenance

Introduction and objective: Affecting millions of people worldwide, cardiovascular diseases represent a significant public health concern. A subset of patients is ineligible for heart transplantation [1]. Consequently, they require the implantation of a circulatory device known as a Ventricular Assist Device (VAD). However, these devices come with certain issues, one of which is thrombus formation. The development of a thrombus within a VAD can potentially lead to a patient's demise. Currently, obtaining a prior diagnosis for thrombus formation that is non-invasive is a relatively complex procedure. Therefore, the primary goal of this study is to create an algorithm capable of replicating time signals that can indicate the presence or absence of thrombus. Subsequently, these signals will be utilized to train Artificial Neural Networks (ANNs) to classify them accurately. **Methodology:** Two primary methodologies were employed in this study. Firstly, we generated the time signals dataset through the utilization of a Signal Reconstruction Algorithm (SRA), incorporating findings from Neto et al. [2], and subsequently, we categorized these signals using an Artificial Neural Network (ANN). This dataset underwent meticulous creation and processing, resulting in the establishment of four distinct classes, each assigned a corresponding label for classification purposes: no thrombus (0), thrombus presence at the rotor base (1), thrombus presence at the rotor vane (2), and thrombus presence at the rotor spiral (3). For the training and validation of the ANN, the dataset was partitioned as follows: 70% of the dataset was allocated for training, while the remaining 30% was reserved for validation. The ANN model utilized in this study was of the Feed Forward (FF) type, featuring three layers. Training involved a total of 4000 epochs, employing the Adam optimizer. The primary metric employed to assess model performance was accuracy. Additionally, the ANN's accuracy was evaluated under conditions of increased background noise in the time signals to further gauge its robustness.
Results and discussion: All the findings initially introduced in the foundational work [2] were methodically reproduced using the Signal Reconstruction Algorithm (SRA). To elucidate the process, the algorithm initiates by reconstructing the time signals, followed by the application of the Fast Fourier Transform (FFT) to derive the power spectra thereof. Subsequently, it proceeds to generate visual representations, enabling a comprehensive visual examination and comparison between the graphs obtained in the original study and the present one.
The Artificial Neural Network (ANN) was then tasked with classifying each of these signals, consistently achieving an impressive average accuracy rate of approximately 91%. It's worth noting that as the background noise levels increased, a discernible decrease in accuracy was observed, further underscoring the network's sensitivity to noise variations.
Conclusions: The identical outcomes that Neto et al. attained might be replicated with some similarity. A mix of signals indicating the presence and absence of thrombus, any spectral background noise distribution, or other intriguing properties can all be easily "synthesized" to create new temporal signals.
Possibility of developing an algorithm that mimics the change from the absence of thrombi to their presence. The next stage will be to develop a predictive algorithm for thrombus detection.

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DEVELOPMENT OF HUMAN FIBROBLAST SPHEROIDS WITH HANGING-DROP INVERTED PLATES

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Keywords: spheroids; hanging-drop; cell culture, 3D

Introduction and objective: 2D cultures have limitations in cell growth. 3D cultures, on the other hand, have become a valuable and powerful tool for biomedical research in recent decades. Due to their resemblance to living systems and cellular interactions, this type of culture can be developed using various methodologies, including nanoparticles, hydrogels, and layers of agarose, among others. Considering the need for testing and validating new molecules and effective therapies for treating various diseases, the objective of this study is to standardize a 3D human fibroblast culture model.

Methodology: HF002-J, human fibroblast cells, were cultured at 37ºC in a humid atmosphere containing 5% CO_2, maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics. When reaching 60-70% confluence, the cells were detached using a 0.05% trypsin solution. Spheroids were prepared using the hanging-drop technique adapted from [1] 440 µL of medium containing cell variations ranging from 2×10^3 to 6×10^4 cells per well of a 96-well plate were deposited, generating a positive meniscus. The plate was inverted and incubated as described.

Results and discussion: The present study aimed to evaluate the development of cellular spheroids after 4 days of culture using different cell preparations. Our results demonstrated that the preparations used produced compact spheroids, characterized by homogeneous sizes in the range of 500 to 1000 µm. When analysing the images obtained by wide-field fluorescence microscopy, we observed that the proportions of unviable cells labelled with fluorophores varied significantly according to the initial number of cells used in the preparations. Notably, increasing the initial number of cells resulted in a proportional increase in the number of non-viable cells present in the formed spheroids. These results suggest that the initial cell density can affect the development and viability of the formed spheroids. It is possible that too high cell density led to greater competition for nutrients and space, resulting in greater cell mortality and less viable spheroids.

Conclusions: Based on the results obtained, it was possible to develop an initial prototype of spheroids from human fibroblast cells that can resemble tissues in vivo due to their cellular interactions, thus providing a new tool for the study of drugs and treatments.

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HIGH-THROUGHPUT PRODUCTION OF TUMOR SPHEROIDS (MELANOMA AND COLON CARCINOMA) USING SIMPLE PLATE TREATMENT AND AUTOMATED FLUORESCENCE MICROSCOPY ANALYSIS

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Keywords: fluorescence microscopy; tumor spheroids; melanoma; colon carcinoma

Introduction and objective: Cancer is currently one of the leading causes of death in the world. The objective of this work is the formation of viable spheroids from cells of melanoma (SKMEL-37) and colon adenocarcinoma (HT29-MTX) cell lines and their evaluation regarding cell viability to enable the use of three-dimensional cell culture as an alternative to the use of experimental animal models.

Methodology: Cells were maintained in RPMI 1640 medium and kept in an incubator at 37°C, 5% CO_2 with controlled humidity. Upon reaching 60-70% confluence, cells were washed with phosphate buffered saline (PBS) and detached using trypsin solution. Afterwards, they were seeded in 24-well plates pre-treated with Pluronic® F-127 (0.5g/mL in 2-propanol) and turned back in incubator for 72 hours. Then, the formed spheroids were stained with Hoechst 33342 and SYTOX® Green solution, incubated for 60 minutes and images were acquired automatically in a HTS equipment (INCell 2500 HS, Cytiva).

Results and discussion: Properly cohesive spheroids were obtained for both lineages, 20-30 per well. After 72h, only a small fraction of cells (about 5%) were considered unviable by SYTOX® staining. Principal Component Analysis (PCA) using 13 variables, and further Principal Component Regression (PCR) showed that nuclei mean and maximum intensities (Hoechst), and nuclei volume are the most relevant variables, corelated to number of plated cells. Days in culture appeared to not correlate with other variables.

Conclusions: It was concluded that the methodology for the production of spheroids for melanoma and colon adenocarcinoma cell lines presented is simple, fast and cheap, in which, in 72 hours, the spheroids form freely, without restriction of shape and size and presenting low cell death, being also compatible with the high throughput screening technique (HTS). Nuclei volume and intensity can be used in future analysis to assess cell global viability in spheroids.

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COMPARISON OF IMAGE DECONVOLUTION METHODS TO IMPROVE IMAGE QUALITY OF 3D CELL CULTURES ACQUIRED USING HCS/HTS EQUIPMENT

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Keywords: 3D cell culture; fluorescence microscopy; image deconvolution

Introduction and objective: Accurate imaging analysis of 3D in vitro cell cultures became critical to evaluation of morphological and some physiological aspects of cells in these aggregates. Fluorescence microscopy protocols allow analysis of a plethora of molecular markers, DNA content and integrity and assessment of morphological changes upon different conditions. Light diffraction by plastic (or glass) substrate, and through sample and mounting/culture media distorts events, and thus images must require deconvolution methods to proper visualization and analysis. The work used 2 different algorithms and 3 iteration numbers to deconvolve images of spheroids acquired in large scale using HTS/HCS equipment.
Methodology: Murine fibroblasts (NIH/3T3) were seeded on non-adhesive 96-well plates in RPMI 1640 medium. After 72 hours in culture, formed spheroids received pan-nuclear (Hoescht 33342) and nucleus of dead cells (SYTOX® Green) staining and imaged using HCS/HTS equipment (INCell 2500 HS, Cytiva). PSF files were generated using fluorescent beads in same cultured medium. Using DeconvolutionLab2 [1], a Fiji [2] plugin, spheroid images were deconvolved using the Tikhonov-Miller or Richardson-Lucy algorithms, with 10, 20 or 30 iterations. Orthogonal views of deconvolved stacks were analysed to find best results.
Results and discussion: Both algorithms rendered similar results, with good reproducibility and resolution of light distortions. No real advantages were perceived in 30-iterations experiments, although 20 iterations rendered best images, regarding shape of nuclei (must be as spherical as possible) and drastic reduction of light refraction between slices.
Conclusions: The work found that both methods were very easy to apply to stacks, with similar results. 20 iterations must be the better option to deconvolve spheroid images, uniting efficiency and saving computational resources. Richardson-Lucy can be the method of choice, as produced slightly better results.

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TOPIC 8

Legal, ethical and regulatory aspects

TOPIC 9

Medical implants and medical devices

MAGNESIUM ALLOY (AZ91) FUNCTIONALIZATION WITH SILICA-GENTAMICIN NANOPARTICLES FOR BIODEGRADABLE IMPLANTS

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Keywords: biodegradable metals; silica-gentamicin nanoparticles; antibacterial effect; temporary implants

Introduction and objective: Biodegradable metals, such as magnesium-based alloys, are extensively studied as temporary implants. These materials must be biocompatible and minimize the release of potentially toxic ions into tissues [1]. Also, as in any surgical procedure, there is a risk of local infection. In order to improve medical treatments, there is a need to locally release antibiotics to avoid systemic doses. Silica-based nanomaterials, usually in the form of spherical nanoparticles, exhibit potential as drug carriers and delivery systems. The objective of this study is to develop and characterize a silica-gentamicin (Si-Ge) system on a pre-treated surface of a magnesium-based AZ91 alloy.
Methodology: Si-Ge nanoparticles were generated employing a modified Stöber method. Samples of AZ91 (9% Al, 1% Zr and Mg balance) polished and cleaned were immersed in a solution with distilled water containing 3.7 g/L $NaHCO_3$ and 0.109 g/L NaH_2PO_4 for 24 h with agitation. Subsequently, 0.1 mL of a 0.02 g/L Si-Ge nanoparticle suspension in ethanol was deposited on each sample. After 24 h, the samples were rinsed with alcohol and sterilized. Each alloy surfaces were characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), particle size (Z-sizer), antibacterial (*E. coli and S. aureus*) and hemolysis assays.
Results and discussion: Si-Ge particles exhibit an average size of 230 nm. After the surface treatment, the alloy observed by SEM appears rough with preferential anchoring sites for Si-Ge particles. FTIR test confirmed that the particles exhibit the typical bondings of silica nanoparticles. The incorporation of species containing C and P from the immersion is also observed on the surface of the AZ91 alloy. They could act as the starting point for a hydroxyapatite formation and hence bioactivity. The hemolysis results yielded values of 32.2 ± 14.1 % for the polished and sterilized alloy, 17.1 ± 4.2 % for the AZ91 with oxide layer and 13.7 ± 1.3 % for the alloy with oxide and the nanoparticles. Although blood-contacting materials ideally should present hemolysis rate below 5% [2], the functionalised AZ91 alloy still demonstrates an improvement in blood compatibility in relation with AZ91 without any treatment. The antibacterial assays showed that after 6 h of exposure to both strains, there is a marked antibacterial activity due to the release of gentamicin, and it remains for 48 h.
Conclusions: The proposed surface treatment was favourable for the anchorage of the nanoparticles with the antibiotic. The presence of Si and P on the surface may induce apatite-like compounds, which is desirable for bone restoration. The treated samples with Si-Ge nanoparticles presented better (but still no ideal) hemocompatibility than the bare material and an excellent antibacterial performance. The proposed modification serves as a starting point for the functionalization of temporary implant surfaces.

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CHITOSAN/JATROPHA MOLLISSIMA-BASED HEMOSTATIC DRESSINGS

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Keywords: Hemostatic; chitosan; *Jatropha* Mollissima.

Introduction and objective: Hemostasis is crucial in surgical procedures as it significantly reduces mortality rates. Chitosan stimulates natural blood coagulation, accelerating cell proliferation and tissue organization, while also exhibiting antimicrobial activities and exudate absorption[1]. This material acts in coagulation, however, its hemostatic capacity is limited. Additionally, *Jatropha Mollissima*, a medicinal plant from the Brazilian Caatinga, possesses antioxidant, antimicrobial, anti-inflammatory, wound healing, and hemostatic properties[2]. Due to the pharmacological importance of these materials, their synergistic effects enhance hemostasis and wound healing. Therefore, this project aims to develop and characterize chitosan-based dressings incorporating *Jatropha Mollissima* for use as hemostatic agents.

Methodology: The sap was collected and extraction was performed using ethyl alcohol (1:1, w/v) at room temperature for 72h. The hemostatic agents were prepared in the form of gel and membrane. The formulations were obtained by dissolving chitosan in 1% acetic acid under mechanical agitation for 1h at 25°C. Then, the dried extract and PEG were added to the chitosan solution and homogenized to obtain the gel. For the membranes, gelatin (5%, w/v) was added to the gel and kept under agitation for 1h, and subsequently subjected to drying in a bacteriological oven at 40°C for 48h.

Results and discussion: The hemostatic agents were evaluated for their physicochemical, mechanical, cytotoxic, and hemostatic properties. For the gels, rheology, injectability, and the presence of substances were analyzed via high-performance liquid chromatography (HPLC-DAD). The gels exhibited pseudoplastic non-Newtonian fluid behavior and solid-like behavior under shear, with increased viscosity at higher temperatures. The injectability assay showed a compression force lower than 30N, indicating suitability for injectable systems. HPLC-DAD identified 8 major peaks characteristic of tannins, phenolic compounds, and flavonoids, quantified as condensed tannins (512.30 mg/g), phenolic compounds (242.84 mg/g), and flavonoids (2.02 mg/g). For the membranes, morphology, thickness, swelling, and wettability were analyzed. The morphology showed a smooth internal surface and a rough external surface, with an approximate thickness of 1mm. The membranes exhibited an average swelling of 164% and a contact angle of 78° decreasing to 63° within 120s, indicating wettability and suitability for use as hemostatic membranes. Both hemostatic materials showed non-toxicity and achieved hemostasis within 3 to 6 minutes, with shorter times compared to the control and commercial hemostatic agent.

Conclusions: The hemostatic agents exhibited physicochemical and biological characteristics within the parameters for use as hemostatic agents. They demonstrated stability at room temperature and within the human body, as well as biocompatibility, showing no toxicity against fibroblasts. The in vivo hemostasis assay demonstrated the induction of a hemostatic effect, reducing the time and amount of bleeding compared to the control group. These results confirm the synergistic action between chitosan and the extract, possibly acting on protein precipitation and an astringent effect.

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USE OF BIOMATERIALS IN REGENERATIVE MEDICINE – OUTPATIENT CELLULAR THERAPY – NEW PROTOCOLS AND EQUIPMENT

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Keywords: COLAOB2023; Biomaterials; Cellular Therapy; Regenerative Medicine; Hydroxyapatite

Introduction and objective: Regenerative Medicine uses the regenerative properties of the body itself. Cellular therapy uses cells, blood derivatives, and bone marrow to promote tissue regeneration with minimal complex procedures. In order to facilitate this regeneration, we are developing new protocols, equipment, and incorporating biomaterials.

Methodology: In Cellular Therapy in Regenerative Medicine, it is common in its protocols to withdraw blood or bone marrow from the patient, processing it then in a centrifuge to facilitate the separation of its components and mainly the stem cells. We demonstrate the possibility of using centrifuges with automatic protocols with complex time and speed profiles, which control the applied G-force differentially, as well as the incorporation of biomaterials such as apatite hydroxide. We will also analyze the automatic separation of these components using the wavelength of light that passes through them and the Bio-stimulation and Bio-modulation through programmable light sources.

Results and discussion: In cellular therapy processes, the differential centrifugation procedures, with intensities and times divided into various stages, make the operation very complex and laborious. The development of a specific equipment, already pre-programmed or with easy and intuitive programming, would allow a significant improvement in practicality, quality, and standardization of the resulting products. The automatic separation of components by wavelength of light, bio-stimulation, and bio-modulation of the products complement the practicality and accuracy of the system, turning a procedure that depended on the practice, skill, and patience of the operator into something standardized. Emphasis will be placed on the use of for wound healing and PRF with Hydroxyapatite for bone regeneration in Dentistry.

Conclusions: The automated equipment and procedures described were developed by the author, while the non-automated equipment and procedures described are currently available on the market. The following protocols were automated: PRP, PRF, L-PRF, A-PRF, i-PRF, and i-PRF+. The i-PRFm protocol was analyzed with Hydroxyapatite.

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CHILD ROBOTIC REHABILITATION SYSTEM WITH ASSESSMENT OF MUSCLE STRENGTH PROGRESSION

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Keywords: Child rehabilitation; cerebral palsy; robotic rehabilitation; bioengineering.

Introduction and objective: Non-progressive ischemic hypoxic encephalopathy, also known as Cerebral Palsy, is the most common disability during childhood. Non-progressive ischemic hypoxic encephalopathy, also known as Cerebral Palsy, is the most common deficiency during childhood. It is characterized by permanent neurological changes that can occur during the prenatal, perinatal and postnatal periods, affecting motor and cognitive development [1]. The increase in muscle strength is a condition primarily sought in the recovery process of motor activity, therefore, it is common to apply resistance to movements during rehabilitation activities. Increased muscle strength is also a strong criterion for evaluating the recovery process. The aim of this work is to develop a system to identify and evaluate the increase in muscle strength through an already developed robotic equipment for child rehabilitation. The idea is that both integrated systems can bring different levels of difficulty during the recovery practice, where the patient advances these levels according to his individual evolution.

Methodology: The equipment used for the application of this work is a teleoperated robotic arm that replicates the movements of the patient's arm captured through inertial sensors present in each part of the arm (arm, forearm, and hand). The intention is for the patient to control the robotic arm in order to perform activities within the scope of playfulness [2]. Therefore, the current methodology starts with the development of a system for capturing and identifying strength levels through electromyographic signals. The idea is to create different levels for the recovery activity, that is, at certain levels, the arm only performs the movement if a certain muscle strength threshold is identified. All results and strength patterns captured can be observed by the therapist through a dashboard.

Results and discussion: The system for capturing and identifying muscle strength levels through electromyographic signals and sensors is effective and the results can be viewed in real time by the therapist through an informative interface. The integration of both devices is currently in the implementation and final adjustments phase, but already showing satisfactory and promising results.

Conclusions: As already mentioned, the project is in its final phase and has not yet been tested in its full development. However, it is possible to glimpse through the results already obtained its full capacity to be applied in a rehabilitation environment, requiring an evolution of the patient's strength capacity and allowing the therapist to analyze this evolution.

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HYBRID STRUCTURES FOR ACHILLES' TENDON REPAIR

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Keywords: tendons and ligaments; hybrid structures; textile technology.

Introduction and objective: Tendons and ligaments are complex connective tissues with unique tensile mechanical properties and complex viscoelastic performance, which is challenging to mimic, posing a considerable healthcare challenge in case of injury, especially in cases where there is significant tissue loss. The present work focuses on the development of implantable structures, with mechanical and degradation properties suitable for the reconstruction of these tissues, and able to promote these tissues' healing rate, to ensure a long-term functional result. For that, hybrid structures composed of poly(lactic acid), PLA, and poly(ethylene terephthalate) (PET), a degradable and a non-degradable polymer, respectively, were studied.
Methodology: Fibrous structures (braids) were developed based on PET and PLA, using textile technology. These braids were then combined into more complex textile structures based on a core-shell architecture. The PET:PLA ratio was optimized to ensure maximum degradability without impairing the mechanical performance of the structure. The structures were characterized by their morphology and mechanical properties (tensile performance, creep, cyclic loading). Surface functionalization with amine groups was applied to the core braids through two approaches: an ethylenediamine (EDA) based treatment and the direct application of NH_3 plasma. Its efficacy was assessed by X-ray photoelectron spectroscopy (XPS).
Results and discussion: The core-shell architecture of the developed structures mimics the hierarchical morphology of the target tissue. PET yarns were determinant for the mechanical strength of the hybrid structures, so braids with higher PET content presented superior tensile performance, good creep, and resistance to cyclic loading, without revealing PLA significant deformation. The porosity of the hybrid structures seems to be enough to cell penetration [1]. The direct application of NH_3 plasma and the surface grafting of EDA after O_2 plasma activation were both effective to introduce amine groups onto the samples' surfaces as demonstrated by XPS analysis. Besides, the plasma parameters chosen do not compromise the topography and tensile behavior of the braids. Both approaches are safe for biomedical applications. The NH_3 plasma approach is more environmentally friendly, faster, and easier to scale up, showing potential for application in a medical device.
Conclusions: An improved structure presenting adequate mechanical performance and degradability with the potential for the repair of the Achilles tendon was obtained. The functionalization of the hybrid braids with amine groups will allow the adhesion and proliferation of cells into the core of the structures for new tissue ingrowth, which along with the PLA degradability in the long term, should enable an adequate biointegration of the hybrid structures.

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COMPARATIVE ANALYSIS OF POLY-L-LACTIC ACID AND CALCIUM HYDROXYAPATITE FOR TREATING CUTANEOUS FLACCIDITY: A CLINICAL TRIAL

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Keywords: biostimulators; dermis matrix; resorbable biomaterials; skin grafts

Introduction and objective: This study presents a comparative analysis between two materials, Poly-L-lactic Acid (PLLA) [1] and Calcium Hydroxyapatite (CHA) [2], regarding their effectiveness in biostimulating the dermal matrix for the treatment of cutaneous flaccidity in four patients. The cutaneous flaccidity, commonly known as sagging skin, is a prevalent concern in aesthetic medicine but quantitative analysis that identify and compare the skin remodelling of dermis is unknown. This research aims to evaluate and compare the biostimulatory properties of PLLA and CHA in the abdominal skin of four female patients, both biomaterials have shown promising results in skin tightening procedures upon injected in abdominal skin.

Methodology: This clinical study assesses the effects of PLLA, CHA, or their combination on the dermal matrix and cells by conducting conventional histology and immunohistochemistry on skin biopsies obtained by 6mm punch. The molecules under investigation include collagen I, elastin, collagen III, and myofibroblasts. Conventional histology will involve processing and staining the tissue sections with H&E, allowing examination under bright-field microscopy. Immunohistochemistry will utilize fluorescent markers, quantified through confocal laser microscopy, to provide additional insights into the molecular changes in the dermis induced by the biostimulation.

Results and discussion: The patients underwent biostimulation using PLLA, CHA, or a 1:1 mixture of both materials, and they are being monitored over time through biopsies taken from each treated area at 0, 30, 60, and 120 days post-biomaterial injections. Prior to each immunohistochemistry evaluation, the histology results revealed distinct characteristics of the dermis at day 0 and 30 post-stimulation. The papillary dermis exhibited a typical double-layer organization and showed a low collagen content. In contrast, the reticular dermis displayed large, unorganized collagen fibers. These findings will present important baseline information about the dermal structure and collagen distribution, which will be further examined and correlated with immunohistochemistry results to gain a comprehensive understanding of the molecular changes in dermis matrix induced by the biostimulation.

Conclusions: This study will provide stronger evidence regarding the behaviour of biomaterials with extracellular matrix stimulating properties, which can support the selection of minimally invasive therapy based on injectable biostimulators in clinical practice. Additionally, it aims to evaluate if there is a synergy process between these materials in vivo. These processes at the tissue architecture level can influence further studies involving other materials, aiming to develop more effective therapeutic options that are economically viable for production and improve patient access to treatment.

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CHARACTERIZATION OF THE *IN VIVO* RESPONSE TO ANODIZED MAGNESIUM ALLOY AS A POTENTIAL BIODEGRADABLE IMPLANT: A 6-MONTHS STUDY

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Keywords: anodizing process; AZ91; biomaterials; Wistar rats

Introduction and objective: Magnesium (Mg)-based alloys are promising candidates for the development of new biodegradable materials for fracture repair [1]. However, in aqueous media, together with Mg degradation, hydrogen evolution occurs, which causes pain and local swelling. Surface treatments, such as anodizing technique, emerge as a potential solution to this limitation. The aim of this study is to understand the long-term performance of Mg-based devices with anodizing as surface treatment that exhibited good short-term (30 days) response both *in vitro* and *in vivo*. However, long-term studies are scarce in literature and are fundamental for defining new biomaterials as safe.
Methodology: Commercial AZ91D alloy was anodized at 5 V, 40 min. Both male WKAH/Hok rat femurs were implanted with anodized AZ91 or PLA (control) pins. After 6 months, femurs were retrieved, fixed and cut. The sections were stained with Toluidine blue for histological examination and histomorphometric analysis of the new bone. Raman spectra were also registered to characterize maturity of new bone. The concentration of magnesium in urine and leukocyte formula in blood samples were measured. Mineralization rate was determined by injecting two fluorochromes into the rats 7 and 3 days before sacrifice and using fluorescent microscopy detection.
Results and discussion: Results showed no hydrogen bubbles at the interface material-tissue. New bone formed around Mg pins was higher in volume (50.3 ± 27%) and trabecular thickness (91.2 ± 44 µm) compared with those around PLA pins (33.9 ± 18% and 71 ± 26 µm, respectively). Ratios of mineral to matrix composition and the degree of carbonate substitution (both indicative of maturation state) of the new bone were calculated based on phosphate, amides and carbonate peaks from Raman spectra. Mg concentration in urine did not differ between groups (Mg pins: 24,1 ± 1,2 mg/dl; PLA pins: 21,8 ± 4,1 mg/dl). Leukocyte formula, indicative of inflammatory processes, did not differ between groups. Mineralization rate could not be determined due to fluorochromes overlapping, and the direction of the mineralization deposition did not present a clear pattern. **Conclusions:** Anodizing process at low voltage is a promising superficial treatment for the development of Mg- based devices for fracture fixation. The *in vivo* response after a long period of implantation is better than the induced by a commercial biodegradable material (PLA) currently used for other applications. Anodized AZ91 promotes bone formation to a high extent and prevents the liberation of hydrogen bubbles without causing negative effects at the systemic level.

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DEVELOPMENT OF A PROTOTYPE POLYURETHANE VALVE RING TO CONTROL MITRAL VALVE REGURGITATION IN DOGS

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Keywords: endocardiosis; biomaterials; polyurethane; valve implants

Introduction and objective: Myxomatous the mitral valve degeneration is the most common heart disease in dogs. It is characterized by a degenerative, progressive, and chronic process, which leads to the weakening of the valve apparatus and, consequently, mitral valve regurgitation [1]. In veterinary medicine, there are few possibilities for curative treatments and drugs only control clinical manifestations. The objective of this project is to develop a prototype valve ring based on polyurethane, which is effective in controlling mitral regurgitation in dogs affected by the disease and allows for greater survival.
Methodology: There is a great variation in the size of the dogs, therefore, in the initial stage, the prototype will be developed from the heart of a pig model. The heart size and the diameter of the valve annulus are measured for the development of matrix prototypes in the Onshape software, with subsequent 3D printing. The matrices are placed in a motor capable of turning 360º for the deposit of polyurethane layers. Finally, the finished prototype will be subjected to cytotoxicity, sterilization, and hydrodynamic performance tests. Animal ethics committee protocol: 2964060723.
Results and discussion: The mean values of the base-apical axis of the hearts, atrioventricular axis of the septal leaflets, and atrioventricular axis of the parietal leaflets were 11.43±0.73 cm, 1.6±0.15 cm, and 1.37±0.06 cm (N=3), respectively. The mean values of the largest and smallest diameter of the valve annulus were 1.13±0.12 cm and 1.63±0.15 cm (N=3), respectively. At this point in the project, the construction of the matrices has started, and we are waiting for the 3D printing. The finished prototype will be presented at this event in December 2023.
Conclusions: Polyurethane has proven to be a very promising material, capable of combining durability, resistance and biocompatibility. This biomaterial has the potential to overcome the limitations of existing mechanical and bioprosthetic valves, as it is less prone to degeneration and calcification, in addition to having very low thrombogenic potential. Thus, it is expected to obtain a prototype capable of sterilization, non-cytotoxic, and with satisfactory hydrodynamic performance [2].

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TOPIC 10

Modeling and simulations

STIFFNESS AND DIMENSIONAL ACCURACY EXPERIMENTAL VALIDATION OF METALLIC 3D PRINTED LATTICE STRUCTURES

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Keywords: Biomaterials; Lattice structures; Compression behavior; Elastic response.

Introduction and Goal: Metallic laser-based powder bed fusion enables sub-millimeter scale 3D printing, allowing the design of biocompatible multi-scale cellular structures applicable in the medical sector for customized bone substitutes and prostheses. The tetrakaidecahedron base cell is a well-known geometry that has been studied in prosthetics due to its similarity to low-density foams and space-filling capacity. This work aims to experimentally validate the dimensional accuracy of printed parts and its effect on the elastic behavior of a tetrakaidecahedron- based microstructure against theoretical designs.
Methodology: Three batches of specimens were designed. Each batch contained five specimens with 5x5 tetrakaidecahedrons in the cross-section and eight tetrakaidecahedrons in height. The batches differ in the orientation of the tetrakaidecahedrons, i.e., they comprised three orientations according to the three orthogonal spatial directions. The batches were manufactured in a laser-based powder bed fusion machine with Ti6Al4V material and 30 µm of layer height. The stiffness of the base material was characterized via nanoindentation testing. Computer Tomography (CT) scans were used to quantify the solid fraction and trabecular thickness distribution. The specimens were tested in compression. FEM models of the test were made to study the effect of dimensional distortions and model boundary conditions.
Results and discussion: The comparison between the CT of printed specimens and the CAD designs shows a solid fraction 15% higher in the physical parts. The measured strut thickness distribution, i.e., trabecular thickness, also differed from the digital design. While a good correlation was found in the first peak at 800 µm, there was a disparity at the second peak. There, the CT scan measured 1000 µm, yet in the digital design, the second peak was 875 µm. These differences are caused by excess material around the struts, particularly on the vertices of the structure, which causes a pseudo-fillet. Based on the CT results, the digital model of the structure was adjusted and analyzed via FEM and compared with a model without geometry deviations. The comparison of both models, considering buckling due to the lack of lubrication in compression, results in a stiffness increase of 10%.
Conclusions: The comparison of results against an asymptotic homogenization proves that the presented specimen design contains enough cells to represent the periodic arrangement of cells. Engineers should note that the dimensional deviations of the printed parts should be considered when designing the microstructure. The induced filleting effect in sharp geometric transitions, such as the vertices of the structure, may impact the stiffness by up to 25% with a marginal effect on the solid fraction. Still, the excess material around the struts increases the solid fraction by up to 15% without considerable impact on the stiffness.

STUDIES FOR THE DEVELOPMENT OF POLYMERIC BIOABSORBABLE STENT FOR COARCTATION OF THE AORTA - CoA

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Keywords: bioabsorbable stent; coarctation of the aorta; geometry of the stent; finite element analysis.

Introduction and objective: Coarctation of the Aorta (CoA) is a congenital heart disease in which stenosis occurs in a section of the aortic vessel, causing its narrowing and decreased blood flow to the body. The use of bioabsorbable stents in newborns will allow the region to be inflated and the stent placed. In this type of stent, the geometry design must provide resistance to support the tensions arising from the aorta and have the flexibility to adapt to complex aortic geometries. The aim is to develop and validate a methodology involving numerical methods and experimental tests for the analysis of bioabsorbable stents.
Methodology: The first experimental studies were about geometry of the bioabsorbable stent. Two models were selected and confectioned with PLA polymer (PolyLactic Acid). The initial stent has a total length of 25 mm, 11 rings in the axis direction, 12 stems in the radial direction, bar-shaped connection elements, an external diameter of 6.75 mm, a thickness of 0.25 mm. Faced with these values it was possible to verify the influence of these geometric changes on the mechanical behavior of the stent, mainly related to the elastic retreat, recoil, shortening, foreshortening and flexibility using Finite Element Method (FEM).
Results and discussion: The experiments were conducted with bioabsorbable stents made by PLA as material for evaluation. Various tests were conducted involving the relationship between pressure and: diameter, length, radial tension and radius.[1] The focus with the tests carried out was to analyze the influence of the stent structure and understand its behavior for a more detailed analysis through standardized tests. Therefore, several test bodies were produced to perform radial expansion, observing their structural conformation using FEM. A CoA model was inserted, and the opening was again simulated. The radial displacement, von Mises pressure in the artery and longitudinal shortening for both stent geometries were measured being the Von Mises tension greater in the center of the stent for both geometry and that maximum filling varied from 3.3% to 3.8% [2]. Comparing the values obtained experimentally with the simulated values, it was noticed that there was a difference of 2.6% in the return of the stent, indicating a good agreement between the numerically simulated values and those obtained experimentally. Being bioabsorbable stent is a factor of great relevance, especially for very young patients who, as they grow up, may again have their aorta narrowed.
Conclusions: The need for the development of bioabsorbable stents for the treatment of CoA is unquestionable. Experimental tests are being conducted to define the best bioresorbable material avoiding problems with pressure and diameter, length and radial tension. These studies showed the great potential that FEM can bring in the development of these kind medical devices.

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COMPUTATIONAL HEMODYNAMICS ON AN AXIAL BLOOD PUMP FLOW

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Keywords: Ventricular assist device; hemodynamic simulation; axial flow blood pump; heart failure

Introduction and objective: heart failure occurs when heart does not fulfil its systolic or diastolic phase correctly. To restore basic or necessary heart conditions, assist devices might be applied, aiming to reach specific goals. In this article, an axial flow pump was adapted, based on [1], followed by a computational hemodynamic simulation with some data from [2] and, by analysing kinetic and dynamic effects, mapping hemolysis critical zones.

Methodology: the pump geometry was based on [1], changing rotor-stator curvature and torsion. Mesh was generated with tetrahedral elements, reaching and independent mesh, used on the simulations. The simulations were performed applying general loop test scenario (5L/min) and testing different rotations scenarios from [1]. In this study, the propeller pitch and its inlet profile were changed, the fluid was considered incompressible, so Cauchy's equations were solved using SIMPLEC method and upwind schemes. Convergence was based on residuals and mean stress calculations, resulting on steady-state solutions after 15000 interactions.

Results and discussion: when analysing the results of the hemodynamical flow for different rotations and discharges, velocity profiles, high/low pressure zones, and streamlines were analysed as a comparison with the initial project and reveal improvements.

Critical zones, where stress overpass hemolysis limit shear stress and recirculating areas, were mapped and analysed. These zones decrease the efficiency of the pump, cause vortices and ma cause unviability in using the purposed device in certain rotations.

Comparisons between rotation increase was also tested and analysed kinetically and dynamically, helping to improve and reach new axial geometries.

Conclusions: hemodynamic behaviour have been improved in relation to [1] geometry. Vortex zones, critical zones and dynamic effects were studied to improve the geometry, reaching more robustness in new geometries.

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ESTIMATION OF TRABECULAR BONE SOLID FRACTION THROUGH ULTRASONIC TRANSMISSION TESTS

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Keywords: Ultrasonography; Trabecular bone; Inverse problem; Biomaterials characterization

Introduction and objective: Estimation of the trabecular bone's solid fraction (BV/TV) is achieved through an inverse problem approach, utilizing ultrasonic wave velocity (SOS) measurements obtained from quantitative ultrasound (QUS) tests. The one-dimensional analytical model developed by Nguyen *et al.* [1] is employed, establishing correlations between six essential bone properties (porosity, trabecular thickness, tortuosity, permeability, stiffness, and microstructural anisotropy) and SOS measurements. The objective of this research is to streamline the Nguyen *et al.* model by reducing the number of variables involved, thereby simplifying the inverse analysis.

Methodology: An analysis of sensitivity based on variance [2] of SOS predictions was conducted on a dataset of 20 million quasi-random samples generated through quasi-Monte Carlo method with Sobol sequences, aiming to evaluate the influence of input variables in the analytical model. Properties with minimal sensitivity were replaced with constant values, while highly sensitive properties were retained as optimization variables. The precision of the simplified model was evaluated by comparing SOS values obtained from simulated QUS using the SimSonic software.

Results and discussion: The variability of permeability and trabecular spacing was found to exert marginal or negligible influence on the model. At low BV/TV values, both the elastic constants and tortuosity show similar influences on the fast wave, while as BV/TV increases, the influence of tortuosity becomes minimal (<0.1 of the total for tortuosity compared to >0.9 for elastic constants). In contrast, tortuosity has the greatest influence on the slow wave (0.9 of the total). At low BV/TV values, a slight interaction between the variables is observed, while at higher BV/TV values, they act practically independently.

Inverse analyses were performed, considering tortuosity and elastic constants as the only variables, without compromising the excellent agreement between predicted SOS from this model and the results of simulated QUS. The differences in calculated SOS between the full and simplified models are negligible, ensuring that the change in model does not affect the outcome of the inverse analysis.

Conclusions: By focusing on the most influential parameters (elastic constants and tortuosity) and neglecting the those with marginal or negligible impact, the model becomes more efficient and easier to work with, without degrading its performance. This streamlined approach not only is a valuable step in optimizing the inverse analysis, making it more practical and reliable for estimating the solid fraction, but also saves time and effort in data collection and analysis of related correlations.

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LARGE EDDY SIMULATION APPLIED TO REACH CRITICAL ZONES IN A CENTRIFUGAL BLOOD PUMP SIMULATION

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Keywords: Ventricular assist device; hemodynamic simulation; large eddy simulation

Introduction and objective: simulating hemodynamics in blood pumps reach different levels of complexity. Turbulence stills an incognito in highly turbulent flows, directly related to critical zones determination [1]. Aiming to analyze critical zones, large eddy simulation models were applied and compared between them and to Reynolds Average Navier-Stokes methods tested by [2].
Methodology: the pump geometry was based on [1 and 2 studies] and the mesh was the same applied on heir studies, which is independent. Upwind schemes and SIMPLEC method were applied to solve Cauchy equations of momentum, considering blood in its Newtonian behaviour, only. It was applied a false-transient analysis, since LES demands time steps, but steady-state flow was intended to analyse. The discharge applied was 5 L/min, the same applied on in vitro loop tests, while rotation was set as 2000 rpm.
Results and discussion: overall pressure difference results show differences between LES and the most accurate RANS model tested by [1]. The comparison between LES simulations demonstrates a range of variation between themselves as well. Critical zones could be presented using LES and a comparison between themselves and the most accurate to the RANS most accurate test by [1] is presented, revealing differences and an overall most sensitive capture from LES model. Kinectic and dynamic effects on LES simulation and velocity profile is also presented, comparing the LES models tested.
Conclusions: large eddy simulation models could be applied to simulate centrifugal blood pumps, although their simulation time and accuracy, compared to RANS models, shows improvement. Critical zones, however, are quite similar, reaching reasonable results.

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EFFECT OF MULTIPLE RELAXATION TIMES IN THE PULSATILE ELECTRO-OSMOTIC FLOW OF BLOOD WITH CHOLESTEROL

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Keywords: Blood with cholesterol; Electroosmotic flow; Transport Phenomena and Rheology

Introduction and objective: The electro-osmotic flow of a viscoelastic fluid between parallel plates is investigated analytically. The rheology of the fluid is described by a new generalized exponential model equation. The charge density obeys the Boltzmann distribution, which governs the electrical double layer field and body force generated by the applied electrical field. The mean objective is to understand the effect of the electrical field in the volumetric flow through the materials, electrical and thermal mechanisms [1,2].

Methodology: The electro-osmotic flow of a viscoelastic fluid between parallel plates is investigated analytically. The rheology of the fluid is described by a new generalized exponential model equation. The charge density obeys the Boltzmann distribution, which governs the electrical double layer field and body force generated by the applied electrical field. Mathematically, this situation can be modelled by the Poisson-Boltzman partial differential equation. By Assuming that the zeta potential is small, i.e., less than 25 mV (Debye-Huckel approximation). Considering a pulsating electric field, the shear viscosity, volumetric flow field and the change in the volumetric flow is presented as a function of the material parameters through the characteristic dimensionless numbers by using an exponential type generalized rheological model.

Results and discussion: Thixotropy, shear thinning, yield stress mechanisms and weight concentration are analyzed through numerical results. Finally, the flow and rheology are predicted using experimental data reported elsewhere for human blood with high cholesterol. The rheological equation of state describes the changes in the structure by effect of the applied forces (tangential and normal) and these forces induced an evolution of the structure (kinetic model) due to the relaxation processes caused by shear strain. It is important to mention that in electro-osmotic flows, complex behavior such as: (i) thixotropy, (ii) rheopexy and (iii) shear banding flow is scarcely explained in terms of the change in the structure of the fluid under flow.

Conclusions: The rheological equation of state describes the changes in the structure by effect of the applied forces (tangential and normal) and these forces induced an evolution of the structure (kinetic model) due to the relaxation processes caused by shear strain. It is important to mention that in electro-osmotic flows, complex behavior such as: (i) thixotropy, (ii) rheopexy and (iii) shear banding flow is scarcely explained in terms of the change in the structure of the fluid under flow.

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APPLICATION OF CYBER-PHYSICAL SYSTEM CONCEPTS TO VENTRICULAR ASSIST DEVICE CONTROL SYSTEMS

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Keywords: Ventricular Assist Devices; Health 4.0; Discrete Event Systems; Cyber Physical Systems

Introduction and objective: Just like any other electromechanical equipment, a Ventricular Assist Device (VAD) is not immune to failures. These can occur due to issues with the hardware or improper interaction with the cardiovascular system. In response to this, our work proposes a safe and innovative controlarchitecture that utilizes Artificial Intelligence (AI) and Discrete Event Systems (SED). This approach is designed to enhance the autonomy and intelligence of the VAD's control system.

Methodology: The proposal is based on Artificial Intelligence (AI) techniques allied to the modelling of Discrete Event Systems (SED). Simulations were carried out to test the efficacy of the proposed control system.

Results and discussion: The simulations revealed reactive control, allowing the VAD to adapt to patient conditions. Furthermore, an embedded computational system allows the creation of a maintenance system that manages routines and a global data information (Big Data), providing diagnostic information to the doctor. With machine learning techniques and the architecture proposed below, it is possible to improve the VAD's control, avoiding recurring failures and enhancing system performance. Thus, VADs align with the trends of Health 4.0 and Hospital 4.0, ensuring connectivity, adaptability, and interoperability within an IoT network.

Conclusions: The proposed control techniques enhance the autonomy, safety, and adaptability of VADs, in line with the trends of Health 4.0. The proposed control system is fault-tolerant and promotes interoperability and connectivity within an IoT network.

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TRIPLY PERIODIC MINIMAL SURFACES SCAFFOLDS, 3D MODELLING AND FINITE ELEMENTS MECHANOBIOLOGICAL SIMULATION

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Keywords: TPMS; scaffolds; Finite elements; bone density growth

Introduction and objective: Triply periodic minimal surfaces (TPMS) models to mimic the biomechanical properties of bone have been used recently more intensively. Showing lot of attention in tissue engineering areas, since additive manufacturing techniques are delivering more challenging geometrical structures with complex pore inter-connectivity arrays, even with the capability to stimulates osteo-induction and osteo-conduction process, geometries which are impossible to build using conventional fabrication methods. Therefore, this work aims the experimental and numerical study of TPMS scaffolds of Titanium Ti6Al4V Alloy, including also, the analysis of bone density growth phenomena induced by stress, and stress shielding effects, using the Finite Elements Method.

Methodology: The scaffolds were modelled using a *gyroid* TPMS, with 300, 400, 500 and 600 µm pore sizes, range of porosities reported as optimal, for cellular colonization (300 µm) and vascularization (600 µm). The geometrical modelling, formed by compression, tension and in-vitro experimental and biological test specimens, considering the porous sizes described above, was performed using MSLattice, Rhinoceros and Fusion 360 software's. All TPMS geometrical models were imported in COMSOL Multiphysics Finite Element software for computational simulations, considering a mechanical and a mechano-biological bone density growth models coupled. The nonlinear plasticity model, was calibrated from experimental tests reported for Ti6Al4V alloy.

Results and discussion: The *Gyroid* TPMS scaffolds with 300, 400, 500 and 600 µm pore sizes were successfully modelled, following a robust methodology developed by our group, guaranteeing the best approximation for the 3D printed geometrical models (.STL archives) and reducing the dimensional error at the microscale of the specimens. The nonlinear Hockett-Sherby and Jonhson-Cook plasticity models, were calibrated from experimental tests reported for Ti6Al4V alloy, for reproducing the specimen's mechanical response submitted to tension and compression. The mechano-biological coupled model, which considers the mechanical constitutive model of plasticity calibrated previously and a biological model bone density growth, developed in previous authors works, have shown its capability to detect zones of bone density growth and resorption due to mechanical loads, for the healthy bone and its post-operative condition after a TPMS scaffold implantation. The bone density growth problem was implemented in COMSOL Multiphysics, considering a Stanford model based on a constitutive approach, including a source term in a mass balance (i.e. continuity equation non-longer zero), in an energy driven format. The mechanical loads, used in the mechano-biological coupled problem corresponds to a femoral rat bone diaphysis physiological environment.

Conclusions: Gyroid TPMS scaffolds with 300, 400, 500 and 600 µm pore sizes were successfully modelled, guaranteeing the 3D printing best approximation and minimizing dimensional errors at microscale. The mechano-biological multiphysics model, considering nonlinear plasticity for the Ti6Al4V TPMS scaffolds and, density growth for biological tissues surrounding, have shown the capability to detect zones of bone density growth and resorption due to mechanical loads, and to simulates the stress shielding effect, showing the potential application of this work, in bone tissue engineering.

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IN SILICO DESIGN OF 3D BIOMIMETIC NANOFIBROUS SACAFFOLDS FOR TISSUE ENGINEERING: DEVELOPMENT OF PARAMETRIC GEOMETRIES FOR EFFICIENT OPTIMIZATION

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Keywords: Scaffolds, Nanofibers, Parametric Geometries, 3D printing

Introduction and objective: 3D printing processing technologies, like Melt Electrospinning Writing (MEW) or Fused Deposition Modeling (FDM), produce highly ordered 3D scaffolds following a G-code file [1]. The development of parametric geometries eliminates the need to design each microstructure separately, enabling the generation of scaffolds with varying microstructure, porosity, and composition by only adjusting the parameters established in the design. Moreover, these virtual geometries can be used for printing the structures but also for modelling their mechanical response by Finite Elements Analysis (FEA) and predicting the optimal combination that results in a biomimetic response [2]. 3D parametric structures using Salome-Meca software are presented.
Methodology: A parametric library of geometries for MEW technology is generated using Salome-Meca software. Different characteristic geometries with different angles and fibers curling degree were selected in order to represent natural tissues extracellular matrices of interest. The parameters selected were fiber diameter, curvature, quantity per layer, angle, among others for the same geometry. As a result, automated CAD design of the scaffolds to be printed with MEW was obtained.
Results and discussion: The focus on developing parametric geometries using Salome-Meca software plays a key role in the project success. The parametric library allows the rapid generation of a wide range of biomimetic nanofibrous matrices with diverse microstructures and compositions. This *in silico* design allow the production of multilayered scaffolds with the same base geometry, as well as a combination of different geometries in other to mimic a more complex tissue microstructure. The 3D scaffold design for MEW processing technology was speeded up significantly, thereby reducing the complexities of the optimization process.
The next step will involve morphology and porosity characterization through scanning electron microscopy (SEM) to study how suitable parameter adjustments faithfully replicate the *in silico* designed geometries.
Conclusions: The development of parametric geometries using Salome-Meca has been instrumental in streamlining the *in silico* design of 3D biomimetic nanofibrous scaffolds. The combination of Salome-Meca and 3D printing technologies has significantly improved the time invested in optimizing the fibrous matrices, enabling swift selection of characteristics for specific tissue engineering applications. This approach represents a promising tool to enhance efficiency and precision in the design of biomedical implants, contributing to the field of biomaterials science.

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ENHANCING PERFORMANCE OF A CATHETER-IMPLANTABLE AXIAL FLOW PUMP THROUGH IMPELLER GEOMETRY ANALYSIS

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Keywords: Impeller, axial flow pump, ventricular assist device, computational fluid dynamics.

Introduction and objective: Ventricular assist devices (VADs) have become crucial in treating heart failure, a global health problem with increasing prevalence. This study focuses on axial flow pumps, a type of VAD that provides continuous blood flow. Despite the benefits of VAD therapy, challenges such as financial costs and device performance remain [1]. Computational Fluid Dynamics (CFD) simulations have emerged as essential tools for evaluating VAD performance and understanding flow patterns [2]. This research aims to analyze impeller geometries of a catheter-implantable axial flow pump using CFD and in vitro tests to enhance pump conditions for improved circulatory support.
Methodology: This study investigates the impact of different rotor geometries on the performance of a catheter-implantable axial flow pump (CIAFP) using computational fluid dynamics (CFD) simulations and in vitro tests. The rotor design parameters, such as pitch value, number of blades, and blade continuity, were analyzed. CFD simulations were conducted using water as the fluid, considering various rotational speeds and flow rates. In vitro tests were performed on a prototype of the CIAFP to compare and validate its hydrodynamic performance.
Results and discussion: The in vitro tests confirmed the accuracy of the CFD predictions, with an average discrepancy of approximately 13%. The results indicate that reducing the blade pitch shifts the performance curve towards with higher head pressures at lower flow rates, while increasing the number of blades raises the overall performance. Conversely, a non-continuous rotor design reduces the hydrodynamic performance for all flow rates.
Conclusions: The results indicated that a low pitch, three-bladed, continuous rotor exhibited the best performance. Further CFD and in vitro tests will be conducted to investigate the interplay between design characteristics and their impact on both hydraulic performance and hemolysis. The ultimate goal is to optimize the CIAFP to maintain the same flow rate and pressure at lower rotational speeds, leading to improved performance.

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TOPIC 11

Nanobiomaterials

DEVELOPMENT OF RADIATION-INDUCED ALBUMIN-BASED NANOPARTICLES

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Keywords: Albumin; Nanoparticles; Melatonin; Nanocarriers.

Introduction and objective: Proteins have been the subject of studies in nanotechnology because they have properties such as high biocompatibility and low toxicity. In this work, we emphasize albumin for the development of nanostructured systems developed by radiation-induced cross-linking [1], intending to deliver melatonin for antitumor purposes, as well as determining its morphological, physicochemical characteristics and evaluating the profile on normal and tumor cells. The development of the study is significant, as new strategies based on nanoparticles make it possible to combat existing challenges in conventional therapy in the treatment of cancer, along with improvements in targeting drugs in the tumor.

Methodology: The synthesis was carried out in the presence of ethanol (30%, v/v) and variation in protein concentration from 0.5 to 10mg/mL in different buffers (phosphate and tris-HCl), both at a concentration of 50mM and gamma radiation (1-20 kGy) for nanoparticle formation. The samples were evaluated using the dynamic light scattering technique assess the hydrodynamic diameter, infrared spectroscopy, and fluorescence. The encapsulation efficiency was made by high-performance liquid chromatography, and the cytotoxicity of the nanoparticles was evaluated for the proliferation in different tumor cells.

Results and discussion: In this work, albumin nanoparticles were developed containing melatonin, aiming for better melatonin availability related to the free drug. The use of radiation for cross-linking of nanoparticles has advantages since it is not restricted to the lack of monomers in the process, and, additionally, the absence of cross-linking agents ensures low residual toxicity and reduces possible purification steps of remaining monomers. Through the characterization analysis of the nanoparticles, it was possible to observe that the average diameter obtained varies from 30-60nm. It was possible to analyze that the increase in the size of the nanoparticles is directly linked to the change of buffer, albumin concentration, and irradiation dose, the average encapsulation efficiency was above 50%, the nanoparticles remained stable for at least 60 days, both at room temperature and in a refrigerated environment. The analyzed nanoparticles did not demonstrate a cytotoxicity profile in healthy cells.

Conclusions: The development protocol of the nanostructured system presented nanoparticles with desirable sizes and stability in the time studied and also observed the efficiency of incorporation of the drug in the nanoparticles, demonstrating promising nanocarriers for the loading of radiopharmaceuticals and physicochemical compatible with the nanostructured systems for application biological.

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NANOCAPSULES BASED ON POLYCAPROLACTONE-TRIOL-BASED POLYURETHANE FOR A DRUG DELIVERY SYSTEM

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Keywords: PCL-T, Piperine, Sustain the release, Nanocapsule.

Introduction and objective: Nanomaterials including nanospheres and nanocapsules show huge potential as drug delivery systems. In recent years, polymeric nanocapsules have attracted more interest in drug delivery applications, taking advantage of their core-shell microstructure. Furthermore, the polymeric shell can isolate the encapsulated payload from the tissue environment, thereby preventing degradation or burst release [1]. Recently, our research group developed a polycaprolactone-triol-based polyurethane (PU) film, which showed hemocompatibility and increased the proliferation of fibroblastic cells [2]. To evaluate the efficiency of piperine encapsulation and release, microparticles of a new PU with three concentrations of piperine.
Methodology: The PU was prepared from the polycondensation reaction between polycaprolactone-triol (300 $g.mol^{-1}$) and 1,6-hexamethylene diisocyanate at 55 °C for 6 h, pure (PU) and loaded with 45 mg (PUPI45), 67.5 mg (PUPI67) and 90 mg (PUPI90) of piperine. The nanocapsules (NPU) were prepared using a simple emulsion method, in which the organic phase was composed of the polyurethane solution and the aqueous phase was composed of polyvinyl alcohol (20 $g.^{L-1}$). The nanoparticles were characterized by DLS and CRYO-TEM (Cryo-transmission electron microscopy). For Cryo-TEM, the samples were prepared in a controlled environment vitrification system, at 22 °C and analyzed using a TALOS F200C (Thermo Fischer Scientific, USA) operating at 200 kV equipped with CMOS camera Ceta 16M 4k x 4k pixels.
Results and discussion: The PU, PUPI45, PUPI67, and PUPI90 showed particle size values of 280.37, 1395.78, 1693.11, and 1237.11 nm; dispersion index of 0.430, 0.072, 0.177 and 0.208; zeta potential of 3.72, -27.11, 4.39 and -39.67 mV, respectively, and despite the increase in particle size, an improvement in system stability was observed, except for PUPI67. The encapsulation efficiency was 80.68±1.75%, 89.23±1.31% and 38.24±1.84% for PUPI45, PUPI67 and PUPI90. Due to the low encapsulation efficiency of PUPI90, piperine release assays were performed for PUPI45 and PUPI67. Given the susceptibility of ester bonds to hydrolysis, the in vitro release of piperine from the PCL-T-based polyurethane conjugate was evaluated at pH 7.4±0.2 and showed a high correlation with the Higuchi model. PUPI45 had a peak piperine release of ~60% at 36 h, slowly decaying and reaching ~30% at 250 h. PUPI67 peaked at ~45% at 144 hours and ~40% at 250 hours. Cryo-EM images were collected for the three compositions (PU, PUPI45, PUPI67, and PUPI90) revealing the presence of nanocapsules with sizes ranging in diameter from 300 to 500nm, an outer monolayer, and a dense core.
Conclusions: The nanocapsules prepared herein can sustain the release of piperine for at least 10 days at room temperature and pH that simulates normal physiological conditions.

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SILVER NANOPARTICLES REDUCED BY TANNIC ACID AND SODIUM CITRATE: A SYNERGIC APPROACH WITH ANTIMICROBIAL PROPERTIES

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Keywords: silver nanoparticles; antimicrobial; green nanotechnology.

Introduction and objective: Green nanotechnology aims to reduce hazardous chemical waste in the environment through sustainable development. Phytochemicals are proposed to minimize environmental impacts and produce safe biological applications. Silver nanoparticles (AgNPs) have effective antimicrobial properties against Gram-positive and Gram-negative bacterias, fungi and viruses, being promising to reduce the microbial load. Antimicrobial systems based on AgNPs shows promise in combating bacteria.
Methodology: Confirmation of the formation of silver nanoparticles was evaluated by UV-Vis spectrophotometry. The hydrodynamic size and polydispersion index were evaluated by dynamic light scattering. The zeta potential was used to assess stability through surface charge. The obtained morphology and average size were evaluated by transmission electronic microscopy. The cytotoxicity assay was performed to assess cellular viability of silver nanoparticles in HUVEC cells. The antimicrobial activity was analyzed by minimum inhibitory concentration through microdilution in broth and later the inoculum was performed in plates.
Results and discussion: Characterization by spectrophotometry of AT_AgNPs and CT_AgNPs showed absorption bands at 430 nm. Hydrodynamic size analyzes revealed diameters of 57.87-97.45 nm, with polydispersion indices (PdI) ranging between 0.289 and 0.392. The zeta potential was determined between -4.41 and -10.3 mV. Transmission electron microscopy (TEM) images revealed spherical morphology with sizes between 20-50 nm. AgNPs have been tested as a treatment against hospital microorganisms with risk classification level 2, including Gram-negative (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria. The Minimum Inhibitory Concentration (MIC) was determined to evaluate the lowest concentration that inhibits the growth of microorganisms. The MIC values obtained with the AT_AgNPs were: 4.18 ug/mL (AB14 and EC23), 8.36 ug/mL (EC26 and KP43), 16.72 ug/mL (PA17), 66.9 ug/mL (PA3) and 133.8 ug/mL (SA). For CT_AgNPs, they were: 51.8 ug/mL (AB14, EC26 and PA17), without inhibition in EC23, KP43, PA3 and SA strains. Synergism was evaluated by mixing suspensions of the two nanoparticles (ATCT_AgNPs), resulting in MIC values of 1.48 ug/mL (PA3 and SA), 2.96 ug/mL (EC23, KP43 and PA17) and 5.93 ug/mL (AB14 and EC26).
Conclusions: The results suggest that AT_AgNPs and the synergistic combination with CT_AgNPs have potential as effective antimicrobial agents against nosocomial bacteria. These AgNPs may represent a promising alternative for the development of new therapeutic strategies to combat bacterial infections. However, further studies are needed to investigate its activity in other contexts and its safety in clinical use.

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OSTEOGENIC EFFECT OF COMPOSITE NANOFIBROUS SCAFFOLDS WITH OSTEOSTATIN

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Keywords: composite scaffolds; nanofibers; biocompatibility; osteogenesis

Introduction and objective: Composite electrospun nanofiber matrices have emerged as promising scaffolds for bone tissue engineering. These matrices offer several advantages, including providing structural support and mimicking the intricate architecture of native tissue, which facilitates cellular growth and tissue regeneration [1]. This study aims to investigate the biocompatibility and osteogenicity of electrospun nanofibrous scaffolds based on poly(ε-caprolactone) (PCL) and hydroxyapatite (nHAp) nanoparticles with and without the surface immobilization of osteostatin (S-O and S). Osteostatin is a pentapeptide 107-111 of the parathyroid hormone (PTHrP 107-111), was used to increase the healing potential of composite scaffolds [2].

Methodology: The scaffolds were electrospun from PCL solutions with nHAp nanoparticles in glacial acetic acid, and later sterilized with peracetic acid and UV radiation . Membranes were inspected by transmission electron microscopy (**TEM**). Osteostatin immobilization in S was performed , obtaining S-O.

In vivo studies; 18 rabbits were divided in 3 groups **A**, **B**, **C** (n=6), A and B underwent surgery for a 10 mm Ø bone defect in each parietal bone of the calvarium: in **A** the left side was implanted with S and in **B** with S-O. In both **A** and **B** groups the right defect did not received any implant. **C** were without any surgery. Clinical, Biochemical, Tomographic studies (**To**) and post-mortem histopathological characterization **(Hi)** were performed.

(0 words).

Results and discussion: Electrospun composite membranes exhibited homogenous nanofibrous morphology. Peracetic acid treatment increased the membranes surface hydrophilicity. **TEM** showed most of the inorganic nanoparticles completely incorporated inside the nanofibers.

There were no intergroup differences for the clinical surveys and biochemical studies carried out during three months. **To** showed that neither A nor B had retraction of the surrounding tissue on the implanted side. And imagens compatible with mineralized tissue were observed, the density being higher in the implanted area of B than of A. **Hi** indicated that no inflammatory infiltrate associated with either S or S-O was observed. In both **A** and **B**, the injured areas without implants, as expected, showed very little new bone formation. **A** in the implanted side with S, showed little new bone formation, both in the center and in the peripheral zone of the implant. **B** in the implanted side with S-O showed spaces with clear new bone formation both in the periphery and in the central zone of the defect. In groups A and B, negative spaces were observed on the injured sides that received implants, corresponding to the biomaterial with associated multinucleated giant cells; this phenomenon was less in group B than in group A.

Conclusions: The findings suggest that the implanted scaffolds, particularly those with that the implanted scaffolds paticulary those with immobilized osteostatin, promote new bone formation and exhibit good biocompatibility in the tested cases. The adequate biocompatibility and osteogenic potential of these materials promote the deepening of new studies to consider their application in regenerative medicine

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ANTITUMORAL EFFICACY OF GOLD NANOPARTICLES WITH POLYPHENOLS IN BREAST CANCER AND METASTATIC CELLS *IN VITRO*

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Keywords: Nanomedicine. Gold nanoparticles. Neoplasia. Tumor Viability.

Introduction and objective: Gold nanoparticles (AuNPs) present beneficial properties in cancer diagnosis and therapy, as they can be coated and biofunctionalized with bioactive molecules through surface modification using relatively non-toxic reagents, enabling the reduction of metal ions, stabilization, and selective detection of cellular receptors [1]. The reduction of gold by phytochemicals to form nanoparticles represents promising methods in Green Nanotechnology. This study aims to compare the binding capacity and evaluate the antiproliferative potential of gold nanoparticles coated with tannic acid (TA-AuNPs) against human breast tumor cell lines. Cytotoxicity was determined using the MTS method.

Methodology: The synthesis of TA-AuNPs was established through the chemical reduction, and the purification method was based on centrifugation, where the centrifuged sample (P1) was resuspended after removing and storing the supernatant (S1), thus generating three samples, including the non-centrifuged (NC). The confirmation of TA-AuNPs formation was achieved by UV-Visible absorption spectroscopy, and the size determination of TA-AuNPs was carried out using dynamic light scattering and Transmission Electron Microscopy (TEM) techniques. The Zeta potential was used to determine the stability of TA-AuNPs. Human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231) were used, and the cytotoxicity determination was performed using the MTS assay.

Results and discussion: The synthesis of TA-AuNPs showed a change in color, indicating the formation of spherical AuNPs. UV-Visible analysis was performed for preliminary characterization, where the absorption band at a wavelength of 529 nm, correlated with the localized surface plasmon resonance band, indicates the presence of approximately 20 nm AuNPs in all samples.

To evaluate the size of TA-AuNPs, the samples were analyzed using the dynamic light scattering method. The hydrodynamic diameters measured indicate that centrifugation induces greater aggregation of the AuNPs. However, based on the polydispersity values ranging from 0.087 - 0.186, the AuNPs showed uniform sizes.

The Zeta potential was an effective indicator used as a criterion to classify and quantify stability, with the samples exhibiting magnitudes above 40 mV, indicating high stability.

TEM images confirmed the presence of TA-AuNPs in the samples and provided information about their dimensions, around approximately 20 nm with spherical morphology.

The cytotoxicity assessment for validating TA-AuNPs demonstrated that the coating plays a crucial role in the degree of internalization, and a higher quantity of specific receptors resulted in a greater internalization of TA- AuNPs. It was observed that there was a higher percentage of cell viability in MCF-7 cells compared to MDA-MB- 231 cells.

Conclusions: The synthesis was efficient in obtaining TA-AuNPs. The TA-AuNPs showed a narrow size distribution, predominantly spherical morphology, and low PDI, indicating significant potential for biomedical applications in the healthcare field. The results satisfactorily and efficiently demonstrated that TA-AuNPs are effective against breast cancer cells (MCF-7) and exhibit higher efficiency in targeting metastatic breast cancer cells (MDA-MB- 231), highlighting their significant potential for various medical applications in the field of nanomedicine.

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BIOLOGICAL CHARACTERIZATION OF SPIONS OF NATURAL ORIGIN FOR POTENTIAL USE IN BIOMEDICAL APPLICATIONS

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Keywords: SPIONs, magnetic hyperthermia, cancer, cytotoxicity

Introduction and Objective: Cancer is characterized as a multifactorial disease, being one of the main pathologies that cause death around the world. In conventional treatment, several adverse effects are frequent, in addition to the high cost. Therefore, the search for alternative therapies with lower incidence of adverse effects and lower cost has been a constant search. Magnetic hyperthermia (MH), using superparamagnetic iron oxide nanoparticles (SPIONs) has shown relevant results. The objective of this work is to verify the biological effect of SPIONs in tumor cells, by *in vitro assays* through the application of hyperthermia.

Methodology: Firstly, a sterility test was performed on samples of SPIONs, then cell viability tests with Trypan blue dye in Vero cells and SPIONs concentration of 25mg/mL in a mirror plate and in 12µl of SPIONs added 288µl of DMEM-HG is added. Afterwards, HM tests were performed without SPIONs and the second experiment with concentrations of 1.0 mg/ml, 1.5 mg/ml and 2.0 mg/ml. MTT was performed in a 96-well plate, adding 100µl DMEM/well. The MTT solution was added to each well and incubated for 4h and the reading was taken at a wavelength of 570 nm in the spectrophotometer.

Result and discussion: The initial results indicate that there is no contamination by microorganisms in SPIONs andthey do not present cytotoxicity in Vero cells in the studied concentrations, in the presence or absence of the magnetic field, also allowed to identify that they are biocompatible nanoparticles [1]. The HM test without the presence of SPIONs, shows an increase in temperature in cells under the action of the magnetic field of up to 30 minutes at 29°C, which does not damage tumor cells, however, in the presence of SPIONs to temperature variation showed variations, as expected, at different concentrations, however, due to the concentrations used, a temperature range between 42°C and 46°C, which is responsible for inducing apoptosis in tumor cells, was not reached [2]. The following steps made it possible to describe the temperature that can trigger apoptosis in tumor cells and to analyze the cytotoxic effects of nanoparticles on tumor cells. In the MTT test, most cells did not survive the triton solution, however, the negative control showed a viability of 30%, even with considerable standard deviations, the results indicate that the HM in the different concentrations used did not cause changes in viability of Vero cells [2].

Conclusions: HH has been a very attractive alternative in cancer therapy. The use of SPIONs of natural origin, in addition to being low cost, low cytotoxicity, is responsible for inducing an increase in temperature in the presence of an alternating magnetic field, which will result in the death of tumor cells by apoptosis. The results point to the use of the technique with promising biomedical applicability.

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DEVELOPMENT OF 3D-PRINTED COLLAGEN SCAFFOLDS WITH *IN-SITU* SYNTHESIS OF SILVER NANOPARTICLES

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Keywords: 3D-printing; UV irradiation method; AgNPs; antimicrobial collagen scaffolds.

Introduction and objective: UV-irradiation method has grown as an alternative approach to in situ synthetize silver nanoparticles (AgNPs)[1] for avoiding the use of toxic reducing agents. Since it is promising the development of materials that could be printed with 3D technology, the aim of this work was to develop a new 3D-printed material based on collagen, with the in situ synthesis of AgNPs in order to endow the biomaterial with the antimicrobial activity of silver.

Methodology: Lyophilized collagen was dissolved with a solution of $AgNO_3$ (0.05 or 0.1 M) to obtain a final collagen concentration of 80 mg/ml. The inks were hydrated overnight and then used to prepare square grids using an extrusion-based Life SI 3D-bioprinter. The 3D-printed scaffolds were irradiated utilizing an UV light (365 nm) during 2, 4, or 6 h at room temperature and then exposed to ammonia vapors to produce gelation of collagen. Col-Ag gels were characterized by SEM, TEM, FTIR and DSC. Swelling capacity, enzymatic degradation, silver content, silver release and antimicrobial activity against Gram-positive and Gram-negative bacteria were studied.

Results and discussion: In this work, an antimicrobial material by in situ synthesizing AgNPs within 3D-printed collagen-based scaffolds (Col-Ag) was developed. By modifying the concentration of $AgNO_3$ and UV irradiation time, the morphology and size of the in situ prepared AgNPs could be controlled. As a result, star-like silver particles of around 23 ± 4 µm and spherical AgNPs of 220 ± 42 nm were obtained for Ag 0.05 M, while for Ag

0.1 M cubic particles from 0.3 to 1.0 µm and round silver precipitates of 3.0 ± 0.4 µm were formed in the surface of the scaffolds at different UV irradiation times. However, inside the material AgNPs of 10–28 nm were obtained. The DSC thermal analysis showed that a higher concentration of Ag stabilizes the 3D-printed collagen-based scaffolds, while a longer UV irradiation interval produces a decrease in the denaturation temperature of collagen. The enzymatic degradation assay also revealed that the in situ formed AgNPs act as stabilizing and reinforcement agent which also improve the swelling capacity of collagen-based material. Finally, antimicrobial activity of Col-Ag was studied, showing high bactericidal efficiency against Escherichia coli and Staphylococcus aureus.

Conclusions: In this work the UV irradiation method and 3D printing technique were combined to in situ prepare AgNPs with different size and shapes, and thus develop broad spectrum antimicrobial collagen-based scaffolds with different thermal, swelling and degradation properties. This is attractive to design and prepare new bactericidal materials by nontoxic and cost-effective methodologies.

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MEDICAL GRADE POLYURETHANE / MWCNT COMPOSITES PRESENTING PHOTOTHERMAL RESPONSE

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Keywords: electrospinning; casting solvent; electroactive materials; photothermal response

Introduction and objective: Photothermal therapy (PTT) is gaining increasing interest as an alternative to conventional therapies due to its specific therapeutic efficacy and non-invasiveness. PTT utilizes photothermal agents that can absorb radiation in the near-infrared (NIR) region and convert the absorbed energy into heat through a non-radiative mechanism. Consequently, nanomaterials with high absorption in the NIR region of the electromagnetic spectrum are required for effective PTT [1,2]. This work aims to obtain and characterize electroactive composites by incorporating multi-walled carbon nanotubes (MWCNT) into the polyurethane with the purpose to investigate the photothermal responsiveness of the composites when exposed to NIR light.
Methodology: The composites were obtained employing two different methodologies: casting solvent (films) and electrospinning (nanofibrous mats) by incorporation of MWCNT ranging from 1 to 3 % with respect to TECOFLEX- 60D. The morphological, thermal, spectroscopic, and surface characterization was carried out using several techniques (electronic microscopies, SEM, DSC, TGA, and contact angle, among others). Finally, using a NIR irradiation device (λ= 850 nm), the photothermal performance of the materials was tested.
Results and discussion: The measured thickness of the films ranged from 500 to 700 µm, while the electrospun mats had a thickness ranging from 500 to 550 µm. The electrospun mats exhibited a uniformly nanofibrous structure, as confirmed by SEM, with fiber diameters ranging from 380 to 520 nm. An increase in fiber diameter was observed with increasing amounts of MWCNTs. All the samples had contact angles greaterthan 70º, indicating their hydrophobic nature. However, the electrospun mats showed higher hydrophilicity compared to the films. The thermal analysis demonstrated the stability of both material types, exhibiting characteristic peaks for polyurethane and confirming the presence of the electroactive nanomaterial. In terms of photothermal performance, a concentration-dependent response was observed. The best-case scenario achieved a temperature increase of over 14°C within 20 minutes of NIR light exposure at a low power density of 90 mW/cm^2.
Conclusions: It is possible to conclude that the obtaining of a family of polyurethane/MWCNT composites in the form of films and electrospun mats was successfully achieved. The properties of the materials allowed for the testing of the photothermal response, which yielded promising results for their potential application in photothermal therapy (PTT). Further studies will be conducted to investigate the nanomechanical properties using Atomic Force Microscopy (AFM) and the inactivation of pathogenic bacteria using PTT.

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DEVELOPMENT OF A NANOPLATFORM BASED ON POLYLACTIC ACID

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Keywords: biopolymer, nanoparticles, polylactic acid, nanoplatform

Introduction and objective: Medical science industry has increasingly focused on researching and developing biocompatible and biodegradable polymers for applications, such as controlled drug administration[1]. However, controlling hydrophobic molecules remains challenging due to their poor solubility in water. Biopolymeric nanoparticles offeran attractive solution, as their large volume-to-surface area ratio and ability to enhance the solubility of pharmaceuticals make them effective for encapsulating and delivering active agents for disease treatment[2]. One promising biopolymer for medical nanoparticle treatments is polylactic acid (PLA), known for its biocompatibility, biodegradability, and excellent mechanical properties[2]. This work aims to develop biocompatible PLA nanoparticles (N-PLA) as nanoplatform for drug delivery.
Methodology: PLA oligomers were previously synthesized with a biocompatible catalyst and characterized by gel permeation chromatography (GPC) and mechanical studies. Subsequently, different concentrations of PLA were tested (5,10,15,20,25 mg/mL solvent) using a nanoprecipitation method. The procedure followed consisted in the addition of oligomers solubilized in acetone (organic phase) added dropwise at 5 mL/h into ultrapure water (aqueous phase). The average hydrodynamic diameter (Dh) was determined at 25°C by Dynamic Light Scattering (DLS) in a He-Ne laser Zetasizer (λ = 633 nm and 173°). The morphology of nanoparticles was investigated using a scanning electron microscope (SEM) Zeiss Sigma.
Results and discussion: PLA oligomers were synthesized using a biocompatible catalyst. The weight average molecular weight (Mw)of the PLA oligomers was 4247 ± 338 g/mol determined by GPC technique. Furthermore, the polymer exhibited a pseudoplastic behavior across a wide range of shear rates, showing a viscosity about of 10^4 Pa*s at 0.05 Hz. These characteristics are highly promising to the formation of nanoparticulate systems.
By employing the nanoprecipitation method, it was observed that increasing the concentration of the polymer led to a growing in particle size. Particles of nanometric sizes were successfully obtained with concentrations of 5mg/mL and 10mg/mL of PLA. The best result was observed at 5mg/mL showing a lower polydispersity index (PDI). This particular concentration of PLA was characterized using DLS and SEM. The N-PLA presented a hydrodynamic radius of 60 nm and a PDI of 0.4. The microscopical study of N-PLA morphology exhibited a spherical shape and smooth surface. The combination of the molecular weight, diameter, and functional groups of the N-PLA presents favorable characteristics to interact with hydrophobic drugs.
Conclusions: It was possible to obtain N-PLA using a nanoprecipitation method in presence of PLA oligomers. The optimal composition of N-PLA was selected and successfully characterized. The particles showed spherical geometry and hydrodynamic radius about 60 nm. The nanoplatform proposed exhibits promising properties for the encapsulation of active agents, particularly hydrophobic drugs.

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ZIRCONIA/BIOACTIVE GLASS NANOCOMPOSITES THROUGH A PARTICLE NANOCOATING FOR DENTAL IMPLANTS

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Keywords: nanocomposites; nanocoating; zirconia; bioactive glass

Introduction and objective: Partially stabilized tetragonal zirconia 3Y-TZP is a promising biomaterial, because of its inertness in biological fluids, good mechanical properties and aesthetics [1]. However, the inertness of this material results in poor biological activity. Cell proliferation and matrix mineralization are of great importance in the process of bone tissue integration with biomaterials [2]. Bioactive glasses are reported to form a strong bond with the tissues and to stimulate bone regeneration. The present investigation proposes a novel route to obtain nanocomposites of 3Y-TZP with a bioactive glass. The objective is to develop dense and homogeneous nanocomposites with high bioactivity and good mechanical properties.
Methodology: Sol-gel technique was used to synthesize new composites, incorporating different percentages of 3Y-TZP powder (YZ), into the sol of a bioactive glass with composition: 64% SiO_2 – 26% CaO – 10% P_2O_5 (mol%) (64S). Different composites were prepared by changing the 64S/YZ ratio. Particle size distribution, SEM and TEM images were performed for powder characterization. Optical dilatometer up to 1450 °C and heating in the range 1100-1500 °C for 2 hours were developed for sintering process analysis. XRD with Rietveld analysis and SEM observation were used for microstructural characterization. Cell adherence and cell proliferation were performed for biological properties characterization.
Results and discussion: Particle size distribution of the coated zirconia powders showed the same bimodal distribution of YZ powder, with a slight shift to higher diameters, suggesting a homogeneous coating. TEM images showed coated-YZ particles with a diffuse layer around the grains of ZrO_2, corresponding to the sol-gel glass coating. Composites started to shrink in the range of 1050-1100 °C, and presented a higher shrinkage than YZ at temperatures above 1100 °C. Complete densification of the composites were obtained in the range 1300-1400
°C. YZ sintered completely at 1500 °C. DRX of the sintered composites showed a principal peak of t-ZrO_2, and the appearance of secondary phases of $ZrSiO_4$ with traces of $Ca_2P_2O_7$. A small amount of m-ZrO_2 was observed in YZ and 64S/YZ samples sintered a 1500 °C. SEM images of the sintered samples showed a dense and pore-free microstructure with a fine grain matrix of t-ZrO_2 (grain size < 1 µm). Vickers hardness of the sintered composites presented high values from 1300 °C (~ 12 GPa), with a slight increase at 1400 °C (~ 13 GPa), comparable with the hardness of YZ at 1500 °C (~ 14 GPa). Cell adherence and cell proliferation improved with the adition of 64S glass.
Conclusions: Sol-gel glass nanocoating of zirconia particles was developed successfully. The composites developed in this study had lower sintering temperatures than zirconia (1300-1400 °C and 1500 °C, respectively). Sintered composites presented high values of Vickers hardness, comparable to zirconia values. Biological activity improved with the addition of 64S bioactive glass. These good results bring a way for further future studies regarding the clinical use of this type of materials.

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MECHANOCHEMICAL SYNTHESIS OF MAGNETIC NANOPARTICLES OBTAINED FROM MINING WASTE FOR BIOMEDICAL APPLICATIONS

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Keywords: magnetic nanoparticles; mechanochemistry; drug delivery; hyperthermia.

Introduction and objective: The disposal of iron mining waste is an environmental problem to be overcome. These wastes contain compounds that can be applied for the synthesis of several traditional and advanced products. Therefore, the objective of this work is the synthesis and characterization of magnetite nanoparticles (Fe3O4) obtained from iron mining waste through a mechanochemical process with a planetary ball mill [1], with potential biomedical applications in cancer treatment and drug delivery.
Methodology: The methodology of this work will initially consist of wet sieving of iron mining waste. Next, high-intensity magnetic separation will be carried out to separate the magnetic compounds in the sample, and low-intensity magnetic separation will be carried out to separate magnetite from other magnetic phases, such as hematite. The magnetite obtained will be processed in a high-energy ball mill to reduce the size of the particles and enable the synthesis of nanoparticles. Finally, the samples will be characterized by: Laser Diffraction Analysis, XRF, SEM, XRD, FTIR, Mössbauer Spectroscopy, VSM, BET and Zeta Potential.
Results and discussion: Roughly 20kg of iron mining waste was cleaned of reddish fines and classified according to NBR 7181/84. Then, the waste was taken to high and low intensity magnetic separation resulting in 26g of magnetite. It is expected that, after grinding in a high-energy ball mill for 24 hours, the sample will reach nanometric size and its chemical, crystallographic, morphological and magnetic properties will be identified.
Conclusions: It is expected to obtain Fe3O4 nanoparticles from an ecological and low-cost route, with sizes and morphology that allows to be applied in cancer theranostics, as well as its functionalization for drug delivery, meeting the international quality standards, such as ISO 10993 that defines standards for evaluating the biocompatibility of medical devices to manage biological risk.

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BIOSYNTHESIS, CHARACTERIZATION AND BIOCOMPATIBILITY OF TIO_2NANOPARTICLES OBTAINED USING *SALVIA ROSMARINUS* EXTRACT

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Keywords: titanium dioxide nanoparticles, biomaterials, green synthesis, *salvia rosmarinus*

Introduction and objective: Green synthesis of metallic nanoparticles (NPs) is an alternative technique to obtain nanomaterials that uses chemical compounds contained in organisms and plants. The biosynthetic route to obtain metallic nanoparticles consists in the chemical bioreduction of metallic salts or precursor metallic oxides, carried by biomolecules with reducing power, allowing the release of metallic ions. These monovalent or divalent metal ions are reduced to metal atoms, forming nanoparticles that can adopt different morphologies. [1, 2] The objective of this study was to obtain titanium dioxide (TiO_2) nanoparticles by biogenic synthesis from natural leaf extracts of rosemary *(Salvia rosmarinus)* for future biomedical applications.

Methodology: The biosynthesis was carried out at room temperature using fresh rosemary leaves extracts and titanium butoxide (IV) as precursor. For the characterization following techniques were performed: Brunauer- Emmett-Teller analysis (BET), Fourier Infrared Transform (FT-IR), UV-Vis Absorbance Spectroscopy and Field Emission Scanning Electron Microscopy (SEM) analysis. For the biocompatibility studies were performed the MTT and neutral red assays in human fibroblasts (BJ) and glial (SVG) cell lines in presence of TiO_2 NPs at different concentrations.

Results and discussion: The NPs obtained has adsorption-desorption type IV indicating the existence of mesoporous with mean pore diameter (2.5 nm), BET surface area (274-370 m^2g^{-1}) and a pore volume (0.23-0.29 cm^3g^{-1}). The FTIR spectra showed peaks in the 450-800 cm^{-1} range corresponding to Ti-O and Ti-O-O stretching vibrations, confirming the formation of TiO_2 NP. Also, the characteristic absorption peak at 380 nm was observed in the UV-VIS spectrum. The SEM images showed a surface morphology spherical. The cell viability in both cell lines was not affected.

Conclusions: The phytocomponents contained in *Salvia rosmarinus* leaves contributes to the obtention of TiO_2 NPs by green synthesis. These nanoparticles are biocompatibles, therefore they could be useful for biomedical applications.

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DEVELOPMENT OF ANTIMICROBIAL FIBROUS POLY(3-HYDROXYBUTYRATE-co-3-HYDROXYVALERATE) – PHBV MEMBRANES LOADED WITH SILVER NANOPARTICLES

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Keywords: Antimicrobial; silver nanoparticle; PHBV ; SBS.

Introduction and objective: Fibers with antimicrobial agents, such as silver nanoparticles (AgNps), are increasingly used in various biomedical applications, including wound dressings and mask materials [1]. The poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a biodegradable, biocompatible and hydrophobic polyester, which can be used as a matrix for dispersing AgNps. Solution blow spinning (SBS) is a technique for producing polymeric micro- and nanofibers, which allows the incorporation of additives. The objective of this study is to produce PHBV fibrous membranes loaded with AgNps through the technique of SBS and "in situ" synthesis of AgNps by UV irradiation. The synthesis of AgNps using green chemistry through UV irradiated is an efficient and sustainable method [2].

Methodology : To produce the polymeric solution, PHBV was solubilized in chloroform at 60°C for 2 hours. Silver nitrate dissolved in dimethylformamide was added. PHBV concentrations used were 3% to 8% (m/v) and $AgNO_3$ 3.5% (m/m). Membranes were produced by SBS using an injection rate of 7.5 $ml.h^{-1}$ and a 0.64 mm diameter needle. Membranes were exposed to the UV irradiation for 10 min to generate AgNps. Fiber analysis involved SEM, TEM, and UV/VIS spectroscopy. The average diameter of fibres and AgNps were evaluated using ImageJ software. The antimicrobial activity was tested against *Staphylococcus aureus* and *Escherichia coli* strains.

Results and discussion: EDS and UV/Vis analysis confirmed the AgNPs formation by UV radiation reduction. Fibers exhibited continuous morphology, but defects were observed in fibers with a concentration of 3% to 5%. Morphological analysis revealed a decrease in defects as the PHBV concentration increased, likely due to the higher viscosity of the solution at higher PHBV concentrations. Fiber diameter did not show significant variation. PHBV fibers with 5% and 6% concentrations containing AgNPs displayed a SPR peak at 438 nm, indicative of nanometric-sized and spherical AgNPs. The 8% PHBV fibers with AgNPs showed four SPR bands, suggesting the presence of AgNPs with different sizes. TEM analysis revealed a uniform distribution of AgNPs, with an average size of 3.58 ± 2.7 nm for the 5% PHBV sample and 8.04 ± 6.7 nm for the 8% PHBV sample. PHBV membranes with AgNPs demonstrated inhibition of bacterial growth against *E. coli* at all tested concentrations. However, they did not show antimicrobial activity for *S. aureus*. This can be attributed to the thicker cell wall of *S. aureus*.

Conclusions: Antimicrobial PHBV membranes were produced by incorporating AgNPs using the SBS technique and UV irradiation. The method is cost-effective, sustainable and suitable for large-scale applications. The average sizes of 3.58 nm (5% PHBV) and 8.04 nm (8% PHBV) were achieved after a 10-minute UV exposure, which is the shortest reported time for direct in-situ synthesis. It was possible to obtain spherical silver nanoparticles with good distribution. The antibacterial activity against *E. coli* increased with decreasing PHBV and $AgNO_3$ concentrations.

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GREEN SYNTHESIS OF TiO_2 NANOPARTICLES USING *OPUNTIA FICUS- INDICA* EXTRACT AND ITS BEHAVIOR IN SIMULATED PHYSIOLOGICAL BODY FLUIDS

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Keywords: TiO_2 nanoparticles; green synthesis; *Opuntia ficus-indica*; physiological fluids.

Introduction and objective: Green synthesis is an alternative method for the bioproduction of nanomaterials using metal salts as precursors and chemical compounds contained or synthesized by living beings, which act as reducing and stabilizing agents[1]. The use of simulated biological fluids (SBFs) *in vitro* solubility assessment of materials is a tool which can allow for a better understanding of the materials behaviour within cellular environments, helping to determine biodurability, bioaccessibility and potentially to predict *in vivo* behaviour[2]. Our objective is to obtain TiO_2 nanoparticles (NPs) through biosynthesis using *Opuntia ficus-indica* as biological phytochemical and evaluate and understand their behaviour in physiological solutions.

Methodology: For the green synthesis of TiO_2 nanoparticles, biocomponents contained in nopal cladiodes obtained by ultrasound-assisted extraction technique and water or ethanol and titanium butoxide (IV) were used. The nanoparticles obtained were characterized physiochemically by Brunauer-Emmett-Teller analysis (BET), Fourier Infrared Transform (FT-IR), UV-Vis Absorbance Spectroscopy and Field Emission Scanning Electron Microscopy (SEM). Stability analysis of NP's was performed in different solutions: phosphate-buffered saline (PBS) and albumin-supplemented PBS, Dulbecco's Modified Eagle Medium (DMEM) and serum-supplemented DMEM and simulate body fluid (SBF) were used at different pH ranges.

Results and discussion: We obtained different mesoporous TiO_2 nanoparticles with BET surface areas between 73.614 and 135.64 $m^2\ g^{-1}$, with a mean pore diameter of 7.65 nm. The vibrational properties of green TiO_2 NP's structures showed that some phytochemicals were absorbed on the surface of this nanoparticles. The vibrations at 1443.4 cm^{-1} for O-H, 1383.8 cm^{-1} for C-OH, 1623.7 cm^{-1} for Ti-OH and 809.9 cm^{-1} for Ti-O demonstrate the presence of carboxylic acids, alcohols or phenols adsorbed on the surface of green TiO_2 NP's. We attributed the difference in surface areas and diameter pore to the parameters that were determined during the green synthesis.

Conclusions: The biocomponents contained in the cladiodes of *Opuntia ficus-indica* can be used to obtain mesoporous TiO_2 nanoparticles by green synthesis. NP's obtained by this method were shown to be biocompatible in simulated physiological fluids.

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DEVELOPMENT OF IMMUNOMODULATORY NANOCOMPLEXES (NANOIMMUNOMODULATORS-NIMs) AGAINST PATHOGENIC BACTERIA

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Keywords: nanoparticles; immunomodulators; bactericidal; co-cultures

Introduction and objective: Silica nanoparticles (SiOHNPs) have multiple properties and functions thanks to their size and their physical and chemical properties, which give them the ability to interact with complex biological functions operating at the same scale as biomolecules. SiOHNPs present good biocompatibility and capacity to transport therapeutic molecules for biomedical purposes. Our objective was to obtain nanoimmunomodulators (NIMs) through the synthesis of SiOHNPs carrying anti-Salmonella *sp.* antibodies and an antibiotic (gentamicin) to activate and help immune system cells against Salmonella *sp.*
Methodology: SiOHNPs were synthesized using the Stöber method [1] and a fraction was chemically modified with APTES and named $SiNH_2$NPs. Size and shape of the NPs were analysed by TEM and microimages were processed by ImageJ software. NPs were also analysed by FTIR and DLS. Anti-Salmonella antibodies (Ab) and gentamicin were immobilized in the NPs by adsorption [2]. Bactericidal activity was tested on two strands of Salmonella *sp*. *In vitro* assays were carried out on two cell line cultures, RAW 264.7 and THP.1 (murine macrophages and human monocytes, respectively) and metabolic activity was evaluated by the MTT assay.
Results and discussion: SiOHNPs with a diameter of 111±11 nm and negative potential (-23.6 mV) were synthesized. The portion chemically modified with APTES showed a positive potential (+13.3 mV). Anti-Salmonella antibodies (Ab) and gentamicin were adsorbed in both variants of NPs obtaining NPs@Ab and NPs@Genta, respectively. NPs@Genta showed bactericidal capacity against two strains of Salmonella *sp*. when used in final concentrations of 600µg/mL, 60 µg/mL y 6 µg/mL while NPs@Ab did not.
In vitro assays with RAW 264.7 cell cultures showed that both NPs@Genta used at high concentrations (300 and 600 µg/mL) decreased the metabolic activity of cells. For SiNH2NPs@Genta, only the highest concentration studied reduced the metabolic activity.
Also, all NPs variables produced plus two mixtures (called Mix): SiOHNPs@Genta + SiOHNPs@Ab and SiNH2NPs@Genta + SiNH2NPs@Ab were tested in vitro with THP-1 cells. At 6 µg/mL, only SiOHNPs and SiOHNPs@Genta didn't alter the metabolic activity of the cells.
Finally, co-cultures were carried out with THP-1 cells and Salmonella *sp.* in the presence of the complexes formed with the negative variants of the NPs at a concentration of 6 µg/mL. After 4h of incubation, cells treated with SiOHNPs, SiOHNPs@Genta and Mix presented a significantly higher metabolic activity (80.35%; 32% and 32% respectively) than the co-culture without NPs.
Conclusions: SiOHNPs@Genta induced a protective effect on the THP-1 against the aggression caused by Salmonella *sp*. This provides a promising tool to be used as NIMs in combination with biomaterials and 3D printing technology for future projects.

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CHITOSAN NANOPARTICLES WITH RUTIN AS A POTENTIAL THERAPEUTIC SYSTEM IN THE TREATMENT OF COVID-19

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Keywords: Chitosan Nanoparticles; Rutin; COVID-19

Introduction and objective: Since the end of 2019, specialists from different areas have started to look for drugs that could inhibit the action of the SARS-CoV-2 virus, which causes the disease COVID-19. Among the various drugs studied to combat SARS-CoV-2, rutin has been identified as a viable herbal medicine against the virus, however, its low solubility makes its usefulness limited [1]. One of the ways to overcome this limitation is by associating it with chitosan nanoparticles. However, no research has been reported on the development of chitosan nanoparticles containing rutin for COVID-19 therapy. Therefore, this work aims to produce and characterize chitosan nanoparticles containing rutin for the treatment of COVID-19.

Methodology: Chitosan/rutin nanoparticles (NP's) were prepared by ionotropic gelation [2]. The chitosan, sodium tripolyphosphate (TPP) and rutin solutions were prepared varying the concentrations between 0.1% and 0.3%. The chitosan and rutin solutions were mixed in a 1:1 ratio and the pH was adjusted to 4.4 by adding NaOH (0.4% m/v) under magnetic stirring. Subsequently, the TPP solution was dripped into the chitosan/rutin solution, in the proportion (1:1), under continuous agitation, for 1 hour, until the NPs hardened. Finally, the NP's solution was centrifuged for 30 minutes at 10 °C and 4000 rpm, to obtain the smallest particles.

Results and discussion: The results showed that the ionotropic gelation technique was efficient for obtaining nanoparticles. The TG of Rutin showed good thermal stability of the drug. The factorial design showed that the TPP concentration was the only influential variable in the process and that the proposed mathematical model can predict with good confidence the behavior of the NP's for the DLS and PZ. The combination of the lowest concentration of TPP, chitosan and rutin resulted in NP's with the best values of Particle Diameter, Polydispersity and Zeta Potential, that is, smaller particle size, better monidispersion and greater stability of the system, respectively, as well as the possibility of better permeability against micrometric systems and greater mucoadhesiveness. Furthermore, the pH of all samples was around 5.35 ± 0.12, close to the pH of nasal secretion (5.5 - 6.5). The FTIR spectra revealed the functional groups of the raw materials and the interaction of chitosan with TPP, indicating the formation of NP's, corroborating with the finding of the spherical morphology of the particles observed in the SEM images. The cell viability assay found that the NP's obtained are non-toxic, in accordance with ISO 10993-5: 2009.

Conclusions: The best results for size, dispersion and stability of NPs were achieved from the lowest concentrations of chitosan, rutin and TPP, demonstrating the possibility of great permeability and good mucoadhesiveness. The formation of NP's was suggested by FTIR and confirmed with the identification of spherical particles observed by SEM. The cell viability assay verified the non-toxicity of the NP's. These data suggest that the NP's obtained were satisfactory, making them promising for their intended purpose.

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GALACTOMANNAN-BASED HYDROGEL AS NANOCARRIER OF α-BISABOLOL FOR WOUND HEALING APPLICATION

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Keywords: Hydrogels; Nanocapsules; Galactomannan; α-bisabolol

Introduction and objective: According to the World Health Organization, worldwide, burns cause more than 180,000 deaths annually, which occur mainly in developing countries. The skin as the largest organ of the human body it assumes multiple important physiological functions and it acts as a barrier to protect against microbial invasion, although it is an organ extremely exposed to burns. However, the development of a sustainable biomaterial to aid in the healing process is challenging and essential. Our goal is to evaluate the physicochemical properties of galactomannan hydrogels containing nanocapsules carrying α-bisabolol for topical application in burn wounds.

Methodology: The methodology used for the preparation of nanocapsule solutions containing α-bisabolol was the interfacial polymer deposition. Firstly, five hydrogels were prepared by solubilizing 1.5% of galactomannan in 10 mL of nanocapsule solution and after being stirred for 2 h at 50 °C. Then 20, 40 and 60% of crosslinking reagent were added to samples 3, 4 and 5 respectively. The nanocapsules and the five formulations were characterized in terms of their physicochemical properties, such as determination of the average diameter (DLS), polydispersion, pH and zeta potential (ZP) of the nanocapsules before and after incorporation into semi-solid suspensions and their rheological behavior.

Results and discussion: The suspension of nanocapsules containing 10 mg/mL of α-bisabolol has an average diameter of 189 nm ± 4 nm and a polydispersion index of 0.08 ± 0.03. Diameter values below 200 nm are capable of releasing their content in the deepest layers of the skin and polydispersity indexes below 0.1 indicate adequate homogeneity of the system.[1] The final pH values of hydrogels formulations were 4,85 ± 0,6 and it is ideal for cutaneous application, since the typical pH of healthy non-diseased stratum corneum surface is around 4.0 to 6.0.[2] The formulation of nanocapsules exhibited negative ZP values of -8 ± 1 mV and -18 ± 4 mV for hydrogels formulations. These ZP values indicate that the stabilization of the system occurs by stereo-hindrance and not by electrostatic repulsion. The DLS analysis also showed that the distribution of diameters of the hydrogel formulations was very similar to the nanocapsule solution, indicating the maintenance of its size after the production of the hydrogels. The rheograms of the hydrogels show that there is no linear relationship between the values of shear stress and shear rate, which configures a non-Newtonian character and pseudoplastic behavior, which is characteristic proceeding for pharmaceutical formulations for topical use.

Conclusions: In summary, it was possible to develop hydrogels that maintained the stability of the average particle size of the nanocapsules containing the active (α-bisabolol). Rheological measurements and gel stability also showed promising results on the use of these formulations for application in burn wounds.

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TOPIC 12

Tissue and organ models

DEVELOPMENT OF MICROFLUIDIC DEVICE FOR ESTABLISHMENT OF THE GLIOBLASTOMA-ON-A-CHIP

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Keywords: Glioblastoma, microfluidic device, alternative model, tumor microenvironment.

Introduction and objective: Glioblastoma is one of the most malignant types of tumors in the central nervous system, yet no effective treatment is available. Our objective is to develop a microfluidic device capable of recreating the structural and physiological organization of the tissue and stroma of glioblastoma. Glioblastoma- on-a-chips could become a powerful tool capable of allowing the evaluation of therapeutic applications in more predictive models of response in humans.
Methodology: Channel design was programmed using CleWin software. The mold was created in SU8 on silicon, and its structures were validated by profilometry. The channels were stamped in PDMS by soft lithography and the inlet/outlet holes were opened, the microdevice was sealed to a glass slide with O2 plasma and finally sterilized (25 kGy). The culture of tumor cells had its viability analyzed at different concentrations of the EHS matrix extract. In Glioblastoma-on-a-chip pre-coated with EHS matrix U87-MG cells were seeded, the medium was changed daily, and cell growth was monitored and after 7 days cell morphology and viability were analyzed. **Results and discussion:** The microdevice developed has a channel with a circular chamber of 2 mm in the central region and two lateral channels of 100 µm positioned in parallel, between these channels the communication is performed through pores of 4 µm thickness and 8 µm in height. The pore size was standardized to obtain the smallest pore size and the best resolution, for a better representation of the in vivo microenvironment [1]. During photolithography, focus correction (-20 µm) was necessary to overcome adhesion problems of SU8 to silicon. The 3D culture of tumor cells in EHS matrix extract was evaluated by confocal microscopy, allowing the observation of better cellular adherence in a short period and more elongated cells with greater intercellular interaction after a period of 24 hours. The SEM analysis allowed the visualization of fibers formed by the EHS matrix, cell-cell, and cell-matrix interactions. We also observed that there was no evidence of dose-dependent toxicity induced by the matrix, but on the contrary, its presence was related to greater viability. Once the ideal concentration of the EHS matrix was determined, the Glioblastoma-on-a-chip had its morphology, cell growth monitored for 7 days, and we observed that there was a compartmentalization of the cells that remained in the central channel of the device and did not cross the pores.
Conclusions: The manufacturing process was successful, being able to build a functional microfluidic device that was able to serve as a basis for cell culture and recreate the tumor microenvironment composed of extracellular matrix and U87-MG tumor cells. Although it is necessary to investigate the use of other matrices and ideal concentrations, the cultivation carried out allowed growth with high cell viability, thus, it was considered appropriate for therapeutic applications.

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DEVELOPMENT AND CHARACTERIZATION OF A LOW-COST DECELLULARIZED AIRWAY SCAFFOLD FOR APPLICATION IN LUNG TISSUE BIOENGINEERING

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Keywords: New approach methodologies, airways bioengineering, decellularization, bronchial tissue.

Introduction and objective: The search for physiologically relevant 3D airway models for in vitro testing has made significant progress in recent decades. The goal is to replace animal-based methods and develop assays that mimic human responses to inhaled toxicants. While some approaches, like 3D bioprinting and commercial hydrogels, have overcome challenges in replicating the extracellular matrix (ECM) microenvironment, reproducing tissue topography and the exact ECM composition remains difficult. This study focuses on creating and characterizing a decellularized porcine bronchial scaffold obtained from food industry waste. The aim is to use this scaffold for reconstructing 3D bronchial models using human cells for long-term cultivation. **Methodology:** Porcine lungs were procured from a nearby slaughterhouse, and fragments from the right and left main bronchi were excised using a circular scalpel. Various matrix decellularization methods (including surfactant solutions, osmotic gradient, and nucleases treatment) were applied to the tissues. Subsequently, the tissues were assessed for characteristics such as tissue morphology, DNA staining, arrangement of collagen fibers, immunolocalization of collagen Type IV, and glycosaminoglycan composition. Finally, the CALU-3 cell line was cultivated on the top of the characterized scaffold, for a 14 days period in an air-liquid interface. **Results and discussion**: The results showed that all the assessed decellularization methods effectively removed tissue cells. However, the SDS 1% treatment and osmotic gradient methods demonstrated the highest preservation of ECM architecture, maintaining the location of collagen Type IV and glycosaminoglycan composition. Additionally, SDS 1% achieved the most effective removal of genetic material from the tissues. Furthermore, the obtained scaffolds were effectively repopulated with the bronchial cell line CALU-3, which was cultivated at the air-liquid interface and exhibited polarization along with consistent expression of airway epithelial biomarkers (MUC5AC and cytokeratin).
Conclusions: The findings demonstrated that surfactant-based decellularization methods can be effectively utilized to produce cost-effective, high-quality extracellular matrix scaffolds for tissue engineering applications. Moreover, the scaffolds exhibited favorable cytocompatibility, enabling the cultivation and differentiation of human airway epithelial cells.

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3D SKIN EQUIVALENTS FOR THE CORROSION HUMAN SKIN MODEL TEST

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Keywords: Collagen I; Bioengineering; Cell migration; 3D Organs Models

Introduction and objective: The development of cell cultures in three-dimensional models is a prominent technology and current challenges include the ability to manipulate cells and biomaterials with the premise of forming structures that mimic the extracellular matrix, simulating natural tissues. This technology is also being explored as an alternative method to the use of animals in testing medicines and cosmetics. The aim of this study is to develop an epithelial tissue synthesised with collagen hydrogel and skin cells, cultured for the production of artificial dermis-epidermis samples to enable animal-free corrosion tests of products for topical use.

Methodology: As the matrix that sustains cell growth reflects on cell performance and, therefore, on the development of 3D skin models, physical-chemical properties of the collagen hydrogel have also been studied. Collagen was characterized by thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR) moreover used as three-dimensional matrix in the development of synthetic tissue, using primary fibroblast and keratinocyte cells, derived from the HaCaT lineage. Initially, fibroblasts and collagen were placed in a transwell insert to prepare the dermis and, after 24 h, the keratinocytes were seeded to simulate the epidermis. After 14 days of culturing, the samples were assessed for cell viability by using the MTT assay and histological analysis, following the guideline OECD of Testing of Chemicals for In vitro skin corrosion: reconstructed human epidermis (RHE).

Results and discussion: Collagen characterization by FTIR and TGA were consistent with those reported in the literature. In FTIR it is possible to identify absorption bands that can be assigned to δ(CH2), δ(CH3), ν(C–N) and δ(N–H) collagen absorption. Absorption of Amides I and II can be found at 1659 and 1555 cm-1, respectively. And the TGA demonstrates the first mass loss close to 80°C and the expressive mass loss was in the temperature range close to 370°C. Histological validation of the synthetic tissue grown on the transwell insert plate showed that in the epithelium formed by this culture method, HaCaT cells initiated the stratification process in the synthetic dermis. After treatment with the selected corrosive material, no cell viability was observed in the MTT assay. In the case of the non-corrosive material, the cell viability was unaffected. Thus, based on these results, the prepared 3D skin equivalent behaved as expected in the corrosion test. Therefore, the reproducibility and repeatability of the artificial epithelium produced on transwell inserts is the key point to validate this study.

Conclusions: In conclusion, this study resulted in a proof of concept for the development of 3D skin equivalents for the corrosion human skin model test. Currently, the reproducibility and repeatability of the results obtained in the corrosion test (OECD Guidelines for Testing of Chemicals, Section 4 · Test No. 431: In vitro skin corrosion: reconstructed human epidermis test method) is being studied, which should result in the validation of this artificial tissue for its application in animal-free testing of topical products.

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