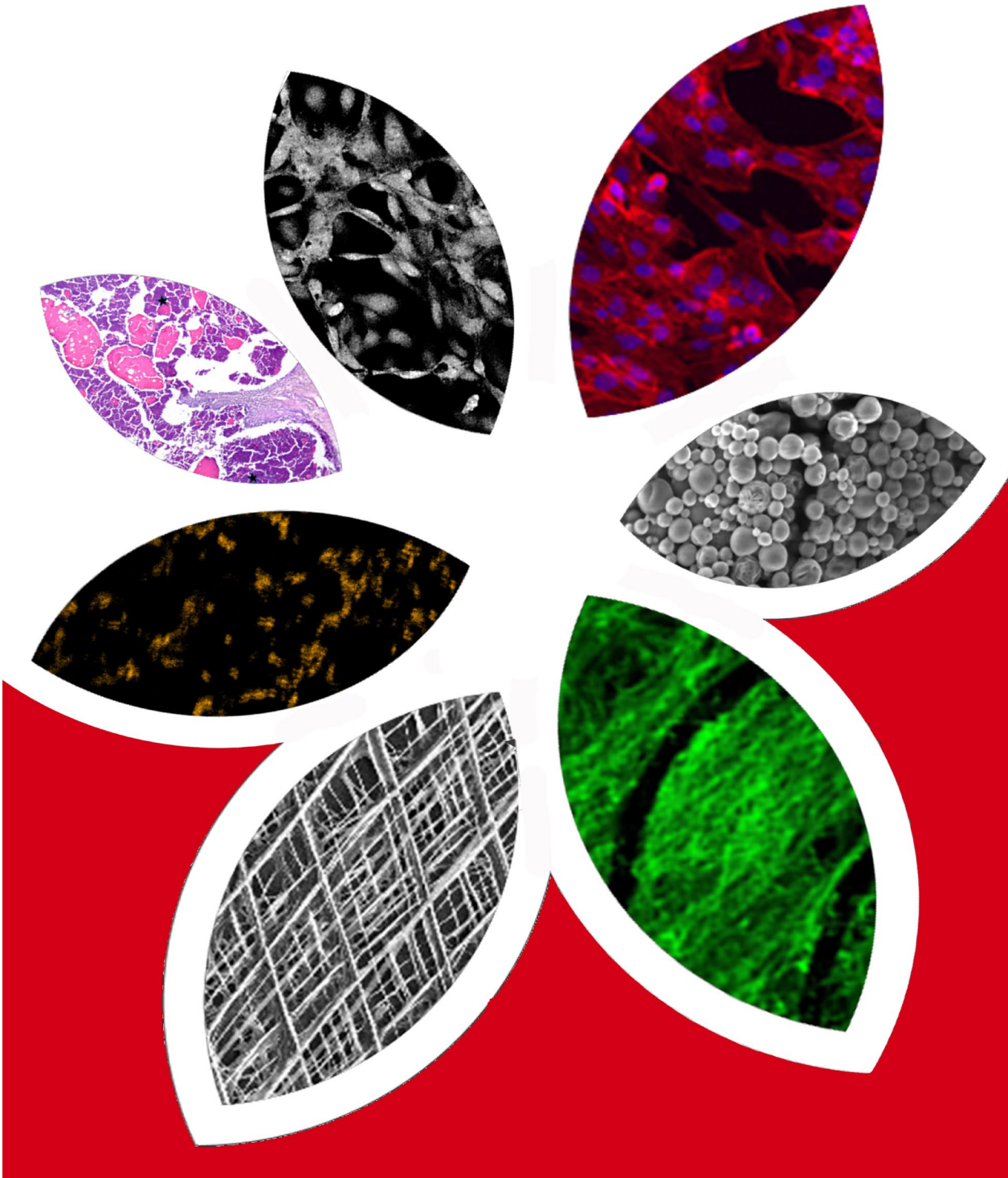
CONFERENCE PROCEEDINGS

POLISH SOCIETY FOR BIOMATERIALS

33rd Biomaterials in Medicine and Veterinary Medicine

Annual Conference

10 – 13 October 2024 Rytro, Poland



33rd Annual Conference
Biomaterials in Medicine and Veterinary Medicine

10 - 13 October 2024 Rytro, Poland

ORGANIZING COMMITTEE

Elżbieta Pamuła - Chair of the Organizing Committee, President of the Polish Society for Biomaterials
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Faculty of Materials Science and Ceramics,
AGH University of Krakow,
Kraków, Poland

WIMiC
WYDZIAŁ INŻYNIERII MATERIAŁOWEJ I CERAMIKI
AKADEMIA GÓRNICZO-HUTNICZA
IM. STANISŁAWA STASZICA W KRAKOWIE

Address of the Organizing Committee

Polish Society for Biomaterials
AGH University of Krakow
Faculty of Materials Science and Ceramics
Department of Biomaterials and Composites
Al. Mickiewicza 30
30-059 Kraków, Poland

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ENGINEERING OF BIOMATERIALS

Open access, peer-reviewed and free of charge journal of the Polish Society for Biomaterials and the Faculty of Materials Science and Ceramics at the AGH University of Krakow issued since 1997

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WWW.BIOMATERIALS.PL

The Journal Engineering of Biomaterials publishes refereed original articles and review papers on biomedical aspects of engineering. It deals with application of materials engineering principles and methods to problems associated with human health. This includes the design and manufacturing of biocompatible materials, implants, artificial organs, controlled drug delivery systems and various medical devices. The journal encourages to present the research results focused on the areas of biomaterials technology and analysis of interaction between implant surfaces and the biological environment/ living tissue to improve the biocompatibility and the biofunctionality of biomaterials.

The scope of the journal includes topics such as:
- Materials Science (Ceramics, Polymers, Metals and Alloys, Composites)
- Biocybernetics and Biomedical Engineering
- Cell & Tissue Engineering
- Biotechnology
- Chemical Engineering
- Nanotechnology
- Surfaces, Coatings & Films
- Medicine
- Veterinary Science

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EDITORIAL OFFICE

Journal „Engineering of Biomaterials”
AGH University of Krakow
Faculty of Materials Science and Ceramics
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30-059 Krakow, Poland
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e-mail: epamula@agh.edu.pl,
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Detailed information concerning manuscript preparation, reviewing procedure and journal policy are available at the journal's website:
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UCZELNIA BADAWCZA
INICJATYWA DOSKONAŁOŚCI

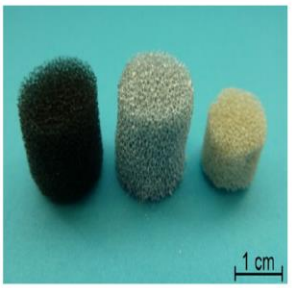
WIMiC
WYDZIAŁ INŻYNIERII MATERIAŁOWEJ I CERAMIKI
AKADEMIA GÓRNICZO-HUTNICZA
IM. STANISŁAWA STASZICA W KRAKOWIE

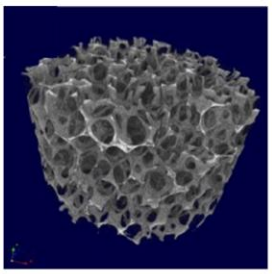
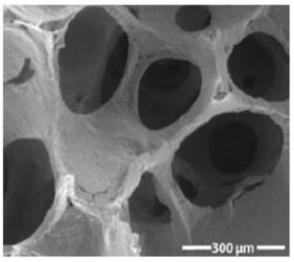
Patronat merytoryczny

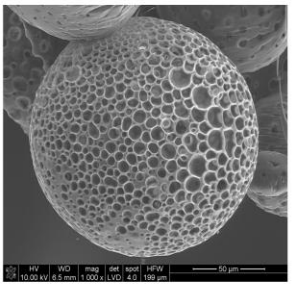
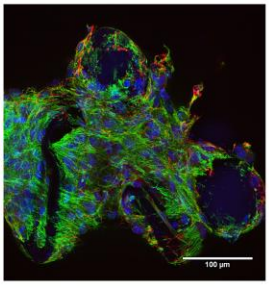
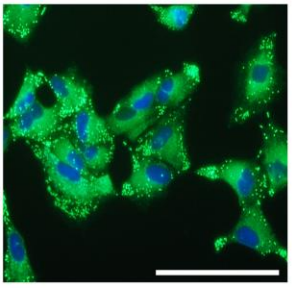
POLSKIE STOWARZYSZENIE BIOMATERIAŁÓW

Studia Podyplomowe
„Biomateriały – Materiały dla Medycyny”

Charakterystyka studiów:
Tematyka prezentowana w trakcie zajęć obejmuje przegląd wszystkich grup materiałów dla zastosowań medycznych: metalicznych, ceramicznych, polimerowych, węglowych i kompozytowych. Uczestnicy zapoznają się z metodami projektowania i wytwarzania biomateriałów, a następnie możliwościami analizy ich właściwości mechanicznych, właściwości fizykochemicznych (laboratoria z metod badań: elektronowa mikroskopia skaningowa, mikroskopia sił atomowych, spektroskopia w podczerwieni, badania energii powierzchniowej i zwilżalności, badania reologiczne, termiczne, mechaniczne) oraz właściwości biologicznych (badania *in vitro* i *in vivo*). Omawiane są także regulacje prawne i aspekty etyczne związane z badaniami na zwierzętach (norma EU ISO 10993) i badaniami klinicznymi, a także najnowsze osiągnięcia inżynierii tkankowej i nanomedycyny.

Termin zgłoszeń: od 20 IX do 20 X
Tryb zgłoszeń: kolejność zgłoszeń
Opłaty: 3 000 zł (za dwa semestry)
Czas trwania: 2 semestry
Zajęcia: osiem zjazdów (soboty-niedziele) raz w miesiącu (AGH Kraków)

Zgłoszenia:

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Pharmaceutics (ISSN 1999-4923) is an open access journal which provides an advanced forum for the science and technology of pharmaceutics and biopharmaceutics. Covered topics include pharmaceutical formulation, process development, drug delivery, pharmacokinetics, biopharmaceutics, pharmacogenetics, and interdisciplinary research involving, but not limited to, engineering, biomedical sciences, and cell biology. The scientific community, the wider community and the general public have unlimited and free access to the content as soon as a paper is published; this open access to your research ensures your findings are shared with the widest possible audience. Please consider publishing your impressive work in this high quality journal. We would be pleased to welcome you as one of our authors.

Polymers (ISSN 2073-4360) is an international, open access journal of polymer science. It publishes research papers, communications and review articles. Polymers provides an interdisciplinary forum for publishing papers which advance the fields of (i) polymerization methods, (ii) theory, simulation, and modeling, (iii) understanding of new physical phenomena, (iv) advances in characterization techniques, and (v) harnessing of self-assembly and biological strategies for producing complex multifunctional structures. Scientists are encouraged to publish their experimental and theoretical results in as much detail as possible.

DevGoMed was created utilizing cooperation of experienced specialists in the field of design, research and implementation into clinical practice of innovative biomaterials and advanced medical devices, including implants. A wide range of assistance in the medical technologies development, DevGoMed relies on experts' many years experience in:

- planning and supervising the medical technology development
- risk management of a medical device along its life cycle
- managing R&D projects
- preclinical and clinical evaluation of medical devices
- implementing a quality management system for the production of a medical device in accordance with ISO 13485
- preparation of technical documentation of products to obtain the CE mark
- post-market surveillance of medical devices

POLBIONICA is a biotech company established by the Foundation on Research and Development of Science to commercialise 3D bionic pancreas research and thinks boldly about the future. The application of the bionic pancreas in clinical practice will revolutionise the treatment of diabetes and become one of the greatest medical successes of the 21st century. Concerning the growing number of patients with type I diabetes and the limitations of available treatments, functional 3D bioprinting is a viable option to overcome the problem of organ shortage and will also reduce the number of complications associated with surgery and the use of long-term immunosuppression after transplantation.
In late 2020, we completed the preclinical phase of 3D bionic pancreas research; we are developing very promising results and we are also preparing for the clinical phase. While working on the 3D bionic pancreas bioprinting project, we created proprietary products crucial for the 3D bioprinting development. These include customised bio inks, a bioreactor, and innovative bioprinting methods.

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34th Biomaterials in Medicine and Veterinary Medicine

Annual Conference

9 – 12 October 2025 Rytro, Poland

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2025

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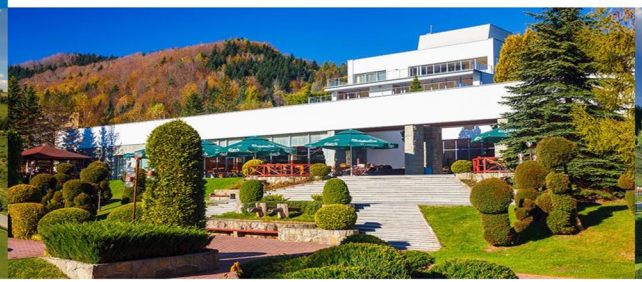
AN ABSTRACT



PROGRAM AT A GLANCE

Thursday, October 10

11:00	Departure to „Perła Południa” from Kraków
13:00 - 15:00	Lunch – Restaurant
15:30 - 15:45	**Welcome and opening remarks**
15:45 - 16:30	**Opening Lecture – Prof. Miguel Oliveira**
16:30 - 17:00	Coffee break
	Session I
17:00 - 18:30	**Oral presentations**
19:00 - late	Regional Dinner – Cottage bar "Nad potokiem"

Friday, October 11

7:00 - 8:00	Swimming pool
7:00 - 8:30	Breakfast – restaurant
	Session II
9:00 - 9:45	**Plenary lecture – Prof. Małgorzata Włodarczyk-Biegun**
9:45 - 10:30	**Oral presentations**
10:30 - 10:40	Global Certification Body – Adam Sobantka
10:40 - 11:00	Coffee break
	Session III
11:00 - 12:30	**Oral presentations**
13:00 - 14:00	**Lunch**
13:30 - 14:15	**Polbionica Workshop**
	Session IV
14:30 - 15:15	**Plenary lecture – Prof. Sachiro Kakinoki**
15:15 - 16:00	**Oral presentations**
16:00 - 16:15	Break
	Session V
16:15 - 16:45	**Keynote Lecture – Dr. Pugalanthi Pandian Sankaralingam**
16:45 - 17:30	**Oral presentations**
17:30 - 18:30	**Poster session and drinks**
19:00 - late	Dinner

Saturday, October 12

7:00 - 8:00	Breakfast – Restaurant/Swimming pool
8:15 - 13:00	Excursion
13:00 - 14:00	Lunch
15:00 - 16:00	**Rapid Fire Presentations – YSF/Poster session**
16:00 - 16:30	Coffee break
	Session VI
16:30 - 18:15	**Oral presentations**
18:15 - 18:30	**Closing remarks and award session**
19:00 - late	Dinner

Sunday, October 13

7:00 - 8:00	Swimming pool
7:00 - 9:00	Breakfast - restaurant
10:00	Departure to Kraków from „Perła Południa”

DETAILED PROGRAM

Thursday, October 10

11:00	Departure to „Perła Południa” from Kraków
13:00 - 15:00	Lunch
15:30 - 15:45	**Welcome and opening remarks – Prof. Elżbieta Pamuła**
15:45 - 16:30	**Opening lecture - Prof. Miguel Oliveira** *Engineering Biomaterials for Biofabrication and 3D in vitro Models*
16:30 - 17:00	Coffee break

Session I chaired by Prof. Małgorzata Włodarczyk-Biegun and Prof. Miguel Oliveira

17:00 – 17:15	Divya Kumar, Marek Bialoruski, Monika Golda-Cepa, Sławomir Lasota, Zbigniew Madeja, Kamil Drożdż, Monika Brzychczy-Włoch, Witold Piskorz, **Andrzej Kotarba** *Functionalization of Carbon Surfaces for Biocompatibility: Oxygen Versus Ammonia Plasma*
17:15 – 17:30	Tayla Ivory-Cousins, Aleksandra Nurzynska, Katarzyna Klimek, Daniel K. Baines, Wieslaw Truszkiewicz, Krzysztof Pałka, **Timothy E. L. Douglas** *Whey Protein Isolate/ Calcium Silicate Hydrogels for Bone Tissue Engineering Applications*
17:30 – 17:45	**Micaela Fernandes**, Julien Es Sayed, Armin Amirsadeghi, Rency Geevarghese, Joanna Żur-Pińska, Marcus Koch, Marleen Kamperman, Małgorzata Katarzyna Włodarczyk-Biegun *Chemically-Induced Jamming and 3D Bioprinting of Granular Hydrogels*
17:45 - 18:00	Michał Fornal, Agnieszka Krawczyńska, **Anna Belcarz** *Accelerated Polycatecholamine Coating of Collagen-Sealed Vascular Prostheses - Comparison of Three Methods*
18:00 – 18:15	**Anagha Mangottukalam Gopalan**, Paulina Chytrosz-Wróbel, Monika Gołda-Cępa, Piotr Kubisiak, Waldemar Kulig, Łukasz Ćwiklik, Andrzej Kotarba *Sonochemical Formation of Ibuprofen Nanoparticles in Water and Ethanol: Towards Controlled Drug Delivery*
18:15 – 18:30	**Konrad Kwiecień**, Karolina Knap, Katarzyna Reczyńska-Kolman, Przemysław Mielczarek, Dorota Ochońska, Daria Niewolik, Katarzyna Jaszcz, Monika Brzychczy-Włoch, Elżbieta Pamuła *Advantages and Limitations of Drug Encapsulation in Polyanhydride-Based Drug Delivery Systems*
19:00 - late	**Regional Dinner – Cottage bar “Nad potokiem”**

Friday, October 11

7:00 - 8:00	Swimming pool
7:00 - 8:30	Breakfast – restaurant

Session II chaired by Prof. Dorota Bociąga and Prof. Sachiro Kakinoki

9:00 – 9:45	**Plenary Lecture – Prof. Małgorzata Włodarczyk-Biegun** *3D Printing for the Reconstruction of Functional Tissue Gradients*
9:45 - 10:00	**Rency Geevarghese**, Joanna Żur-Pińska, Małgorzata Włodarczyk-Biegun, Daniele Parisi *Bioink Designing: Balancing Printability, Degradability and Bio-Compatibility*
10:00 - 10:15	**Katarzyna Reczyńska-Kolman**, Dorota Ochońska, Kamil Kornaus, Monika Brzychczy-Włoch, Elżbieta Pamuła *Antibacterial and Osteogenic Calcium Carbonate-Based Microparticles for Bone Tissue Regeneration*
10:15 – 10:30	F. Porzucek, M. Mankowska, J. Semba, P. Cywoniuk, A. Augustyniak, A. Mleczko, Ana Teixera, Pedro Martins, A. Mieloch, T. Szymański, **Jakub Rybka** *Development of a Porcine Decellularized Extracellular Matrix (dECM) Bioink for 3D Bioprinting of Meniscus Tissue Engineering: Formulation, Characterization and Biological Evaluation*
10:30 – 10:40	**Adam Sobantka** **Sources of Financing for Development and Commercialization of Products**
10:40 - 11:00	Coffee break – Poster viewing

Session III chaired by Prof. Anna Belcarz and Prof. Andrzej Kotarba

11:00 – 11:15	Alexandra E. Snyder, Jada K. Sandridge, Adeline E. Nordmoe, Evan N. Main, **Gary L. Bowlin** *Fabrication and Mechanical Characterization of Near-Field Electrospun Bioresorbable Vascular Grafts Mimicking the Arterial Extracellular Matrix*
11:15 – 11:30	Krzysztof Galbas, Josef Spreitz, Roman Kustosz, **Małgorzata Gonsior Kustosz** *Poli-IN - System for Closing an Inefficient Saphenous Vein - from Idea to First Clinical Application*
11:30 – 11:45	**Roman Major**, Karolina Szawiraacz, Marcin Surmiak, Maciej Gawlikowski, Przemysław Kurtyka, Aneta Dyner, Marcin Basiaga *Evaluation of The Biological Suitability of Metamaterials Dedicated to Atraumatic Laparoscopic Instruments*
11:45 – 12:00	**Witold Łojkowski**, Urszula Szałaj, Julia Higuchi, Olena Sych, Wojciech Majewski, Svetlan Stelmakh, Elżbieta Pietrzykowska *Nanoarchitecture Based Orthopaedic Implants Using High Pressure, Ultrasonic and Microwave Technology – Towards Clinical Tests*
12:00 – 12:15	**Oleksandra Pryshchepa**, Paweł Pomastowski *Ferrite Magnetic Nanoparticles (Fe_xO_y) in Biomedical Science: Breakthroughs in Protein Fractionation and Isolation*
12:15 – 12:30	**Pavan Kumar Reddy Gudeti**, Taha Cagri Senocak, Piotr Stanisław Zieliński, Marcus Koch, Małgorzata Katarzyna Włodarczyk-Biegun *Enhanced Tendon Regeneration through Biomechanical Approaches: Additive Manufacturing and Mechanical Stimulation*
13:00 – 14:00	**Lunch**
13:30 – 14:15	**Workshop (Hotel Lobby): *Preparation and Culture of Bioprinted Tissue Models - Presentation of Bioreactor 3D Flow&Culture Device***

Session IV chaired by Prof. Beata Świeczko-Żurek and Prof. Gary Bowlin

14:30 – 15:15 **Plenary Lecture – Prof. Sachiro Kakinoki**
Peptide Immobilization to Create Bioactive and Bioinert Surfaces for Blood-Compatible Medical Devices

15:15 - 15:30 **Marcin Kaczmarek**, Natalia Słomian, Witold Walke, Zbigniew Paszenda
Suitability of Heat-Treated Co-Cr-Mo Alloy Intended for Applications in Cardiology

15:30 - 15:45 **Przemysław Kurtyka**, Sachiro Kakinoki, Maciej Gawlikowski, Angelika Auguścik, Justyna Więcek-Chmielarz, Karolina Szawiraacz, Klaudia Cholewa, Artur Kapis, Agnieszka Szuber, Jürgen M. Lackner, Roman Major
Bioneutral, Hemocompatible Oligoproline Coatings Evaluation Towards Application in Polish Heart Support Systems for Children

15:45 - 16:00 **Daniel K. Baines**, Varvara Platania, Mattia Parati, Karen Wright, Izabela Radecka, Maria Chatzinikolaidou, Timothy E. L. Douglas
Whey Protein Isolate/Poly-γ-glutamic Acid Hydrogels Promote the Proliferation and Osteogenic Differentiation of Pre-osteoblasts

16:00 – 16:15 Break

Session V chaired by Prof. Aleksandra Radtke and Prof. Janusz Szewczenko

16:15 – 16:45 **Keynote Lecture**
Pugalanthi Pandian Sankaralingam, Vijayakumar Chinnaswamy Thangavel, Nandhini Pandian
Metal Fluorophosphate Based Nanopowders for Wound Healing

16:45 - 17:00 **Andrzej Swinarew**, Jadwiga Gabor, Paweł Raif, Grzegorz Brożek, Agnieszka Jarosińska, Jan E. Zejda, Szymon Skoczyński, Jarosław Paluch, Natalia Brzezińska, Arkadiusz Stanula
Novel Carbon-Based Materials in Exhaled Air Metabolome Analysis for Childhood Asthma Fingerprints Identification

17:00 – 17:15 **Marta Klak**, Sylwester Domański, Dominika Ujazdowska, Milena Czajka, Małgorzata Popis, Oliwia Janowska, Magdalena Dec, Katarzyna Florys-Jankowska, Katarzyna Kosowska, Michał Wesołowski, Tomasz Dobrzanski, Andrzej Berman, Michal Wszoła
From Multiwell Plates to Bionic Organs. Step Forward to Perfusable 3D Tissue Models

17:15 – 17:30 **Gabriela Gąsior**, Aleksandra Radtke, Tomasz Jędrzejewski
Porous Iron-Based Materials for Use as Cardiovascular Implants - Biological and Chemical Properties

17:30 - 18:30 Poster session and drinks

19:00 - late Dinner

Saturday, October 12

7:00 - 8:00	Breakfast – restaurant/Swimming pool
8:15 - 13:00	Excursion
13:00 - 14:00	Lunch
15:00 - 16:00	**Rapid Fire Presentations – YSF**
16:00 – 16:30	Coffee break
Session VI chaired by Prof. Krzysztof Pałka and Dr. Timothy E.L. Douglas	
16:30 - 16:45	**Patrycja Domalik-Pyzik**, Malwina Furgała *Hollow Alginate Capsules Modified with Silver and Copper Nanoparticles for Theranostic Applications*
16:45 – 17:00	**Anna Taratuta**, Karolina Goldsztajn, Julia Kolasa, Julia Lisoń-Kubica, Magdalena Antonowicz, Marcin Basiaga *Characteristics of Silicon Oxide Thin Films Prepared by Electrophoretic Deposition Method (EPD) for Implants for Cardiovascular Applications*
17:00 – 17:15	**Karolina Goldsztajn**, Marcin Godzierz, Maciej Sowa, Katarzyna Nowińska, Anna Hercog, Joanna Jaworska, Katarzyna Jelonek, Wojciech Kajzer, Roman Major, Janusz Szewczenko *Physical and Chemical Properties of Sandwich-Type Poly(D,L-Lactide-Glycolide) Coating Containing Hydroxyapatite and Dexamethasone on Titanium Alloy Substrate*
17:15 – 17:30	**Agnieszka Antończyk**, Magdalena Antonowicz, Witold Walke, Justyna Majewska, Karolina Goldsztajn *Evaluation of The Physicochemical Properties of PMMA-based Spherical Aluminosilicates*
17:30 – 17:45	**Mareeswari Balasubramanian**, Iwona Pudełko-Prażuch, Sundara Moorthi Ganesan, Stanisław Marecik, Kamila Walczak, Kinga Pielichowska, Suvro Chatterjee, Ravichandran Kandaswamy, Elżbieta Pamuła *Porous Scaffold - Transforming with Blends*
17:45 – 18:00	**Sundara Moorthi Ganesan**, Mareeswari Balasubramanian, Ravichandran Kandaswamy, Pugalanthi Pandian Sankaralingam, Vijayakumar Chinnaswamy Thangavel *Scaffolding of a Composite for Bone Tissue Engineering*
18:00 – 18:15	**Closing remarks and award session**
19:00 - late	Dinner

Sunday, October 13

7:00 - 8:00	Swimming pool
7:00 - 9:00	Breakfast - restaurant
10:00	Departure to Kraków from „Perła Południa”

LIST OF POSTERS

1. **Anna Kłeczek**, Jadwiga Gabor, Jarosław Paluch, Robert Kwiatkowski, Andrzej Swinarew
   Innovative Application of Porous Carbon Material for Detection of Laryngeal Cancer Through Exhaled Breath Analysis
2. **Maciej Pyza**, Artur Sowiński, Ludwik Tarachowicz, Jadwiga Gabor, Andrzej Swinarew
   Composites Containing Plant Origin Carbon in Emergency Medicine and Water Rescue Applications
3. **Dominika Wójtowicz**, Jakub Pustułka, Łukasz Zych, Agnieszka Lechowska-Liszka, Anna Ścisłowska-Czarnecka, Ewa Stodolak-Zych
   Studies on Biocompatibility and Permeability of Nanocomposite Dialysis Membranes Modified with Carbon Nanoforms
4. **Kotomi Kitada**, Sayuki Yoshitomi, Taiki Morishige, Sachiro Kakinoki
   Surface Modification of Pure Magnesium to Inhibit Early-Stage Rapid Corrosion and to Improve Osteoblast Compatibility
5. ***Barbara Rynkus**, Maciej Sowa, Janusz Szewczenko, Wojciech Simka*
   Corrosion Behavior of Ca-P Coating Prepared on Magnesium Alloy by Plasma Electrolytic Oxidation
6. **Karina Niziołek**, Dagmara Słota, Agnieszka Sobczak-Kupiec
   Formation and Characterization of Calcium Phosphate Cements Modified with Magnesium Ions for Bone Regeneration Applications
7. **Janusz Szewczenko**
   Degradation of Anodic Oxidized Titanium Alloys Under Conditions of Electrostimulation of Bone Union
8. **Joanna Zontek-Wilkowska**, Agata Krakowska, Przemysław Dorożyński, Rafał Kaim
   Ozonation Process as an Effective Method for Desinfection and Sterilization of Textiles
9. Agata Krakowska, **Joanna Zontek-Wilkowska**, Piotr Szatkowski, Przemysław Dorożyński, Bożena Muszyńska
   Biomass From In Vitro Cultures - Its Biomedical Potential
10. **Dorota Bociąga**, Jacek Grabarczyk, Piotr Niedzielski, Michał Bogdański, Mohanraj Shanmugam
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RAPID FIRE PRESENTATIONS
PART OF THE BEST POSTER COMPETITION

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3D PRINTING FOR THE RECONSTRUCTION OF FUNCTIONAL TISSUE GRADIENTS

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Introduction

Native tissues are typically highly organized structures with a complex cellular microenvironment. They are non-uniform and often display functional gradients in both architecture and biochemical composition [1]. Regenerating these complex structures after damage caused by injury or disease is generally challenging and may not fully restore the original tissue functionality.
Therefore, our research focuses on extrusion-based printing techniques, such as bioprinting and melt electrowriting (MEW), to engineer multilayer scaffolds that closely replicate the structure and function of hierarchical native tissues. Specifically, we employ MEW for the reconstruction of the Human Trabecular Meshwork (HTM; a membrane in the eye) [2], hard-soft tissue interfaces (like the junction between muscle and tendon) [1], and skin tissue [3]. We combine MEW structures with custom hydrogels, and to advance toward functional solutions, we propose new printable formulations that offer enhanced adhesiveness, biocompatibility, and resolution.

Materials and Methods

Melt electrowriting of medical-grade polycaprolactone (PCL, PURASORB PC 12) and novel elastomer was performed with dedicated printers (Spraybase, Ireland, Bioscaffolder, GeSiM). 3D extrusion printing of developed soft materials was done using Bioscaffolders (GeSiM or Felix). PCL scaffolds with different designs (varied pore shapes and sizes, different number of layers), including gradients, were printed and visualized with scanning electron microscopy (SEM). Bioinks based on granular hydrogels and coacervates were developed and printed.
Obtained scaffolds' mechanical (in tensile and compression modes) and biological properties (cell viability, activity and differentiation) were characterized. The relation between structure and function was analysed.

Results and Discussion

Different designs (homogeneous and gradient) of the fibrillar scaffolds were proposed and successfully printed (FIG. 1). Some of the geometrical features closely mimicked the native architecture of particular tissues (i.e., skin, tendon, or HTM). Mechanical testing revealed the influence of the design on the mechanical properties of the scaffolds. Additionally, the MEW scaffolds enclosed in the hydrogel matrix were characterized by higher stiffness and toughness. Fibroblasts and primary HTM cells attached to the scaffolds, proliferated, spread, bridged the pores, and maintained their phenotype. Cell behaviour was influenced by the orientation and material properties of the MEW fibres (FIG. 1).
Additionally, stimuli-responsive (with sensitivity to the temperature, pH, and salt concentration) soft printable materials were successfully developed. They exhibited excellent printability and specific functionality beneficial for patient-specific tissue engineering.

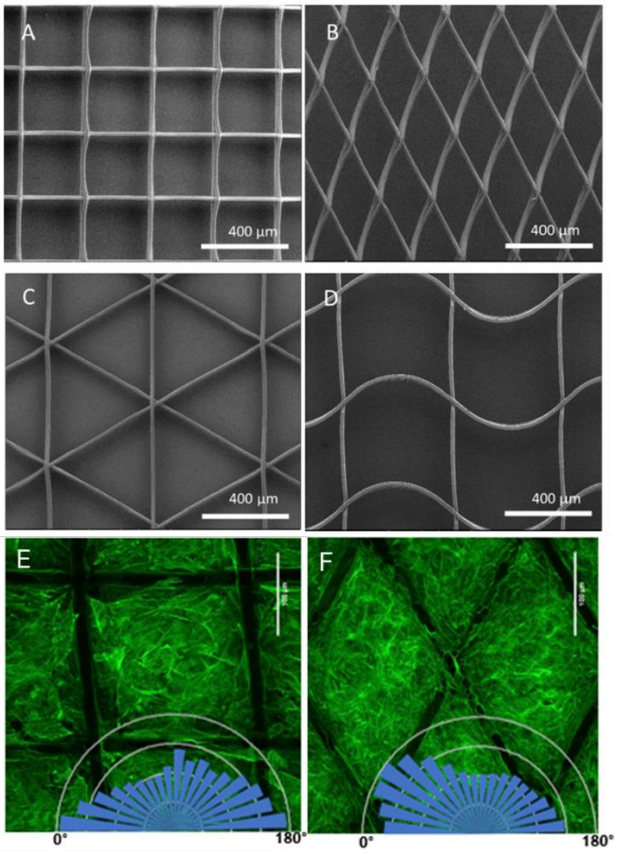

FIG. 1. SEM images of exemplary printed designs using the MEW approach (A-D). Primary human fibroblasts growing on different printed patterns with specific orientations (E-F).

Conclusions

We have demonstrated that biofabrication, particularly the MEW technique combined with hydrogel deposition, is an effective method for creating small-scale scaffolds with high precision. Printed designs can be customized for specific applications. Additionally, we successfully developed hydrogel-based printable materials with exceptional stimuli-responsive properties.
Developed approaches have allowed us to reconstruct the hierarchical structure of hard-soft tissue interfaces, HTM, and skin. These hybrid scaffolds, with their complex biomimetic architecture, hold potential for future use as implantable systems or as in vitro testing models.

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PEPTIDE IMMOBILIZATION TO CREATE BIOACTIVE AND BIOINERT SURFACES FOR BLOOD-COMPATIBLE MEDICAL DEVICES

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Introduction

The surfaces of medical devices are directly exposed to their surrounding environment, and therefore, they play a crucial role in determining key properties such as durability and functionality. Upon implantation in the body, the interactions that occur within the first few seconds between the device surfaces and biological components, including proteins, cells, and blood, determine the long-term success or failure of the implantable medical devices. Thus, the technology for designing surfaces that regulate biological phenomena should play an essential role in determining the fate of implantable medical devices. There are two strategies for avoiding unfavorable foreign body reactions to medical devices: bioactive and bioinert. Bioactive means the approach to improve the interaction with specific components to lead the favorable biological responses. On the other hand, bioinert refers to the property that allows the body to ignore the medical device, enabling it to function without causing any harm or triggering any biological responses. This talk will discuss the usability of peptide immobilization technology in creating bioactive and bioinert surfaces, especially for blood-compatible medical devices.

Materials and Methods

Bioactive: Endothelialization promoting surface

Peptides composed of an anchor sequence and fibronectin-derived cell adhesive ligand (LDV), Ac-$(YK)_3G_3$LDV was synthesized and immobilized on ePTFE films through the direct Tyrosine oxidation with copper catalyst and hydrogen peroxide [1-3]. After characterizing its surface properties, the adhesion behavior of endothelial cells was evaluated *in vitro*. In addition, the neointimal-like tissue regeneration *in vivo* was investigated using a small elliptic ePTFE patch immobilized Ac-$(YK)_3G_3$LDV for closing a hole made on the rat carotid artery (Approval #1805, Kansai Univ.). Furthermore, the surface co-immobilized with integrin α_{11} ligand and heparin was also prepared by the cross-linking layer-by-layer method and its biological functions were evaluated.

Bioinert: Anti-biofouling surface

Collagen backbone-inspired peptides, oligoprolines (Ac-Cys-Pro_n-NH_2, n= 6 or 9) were immobilized on the Au-sputtered coverslip through Au-SH bond. Oligoproline-immobilized (OP) surfaces were characterized by the water contact angle measurement and X-ray photoelectron spectroscopy (XPS). Subsequently, protein adsorption, fibroblasts, platelets, blood cells, and bacterial adhesion on OP surfaces were evaluated [4, 5].

Results and Discussion

Bioactive: Endothelialization promoting surface

After the reaction in solution of Ac-$(YK)_3G_3$LDV, peptide-derived nitrogen (N1s) was detected on ePTFE surfaces by XPS analysis. The nitrogen peak did not disappear after washing by both 1.0% SDS and 1.0 M NaCl aqueous solutions, indicating that Ac-$(YK)_3G_3$LDV might form a stable layer on ePTFE through adsorption and polymerization of $(YK)_3$ anchors. This is because quinones produced by the oxidation of hydroxyphenyl groups triggered phenol coupling and Michael addition. Adhesion of endothelial cells was significantly improved on the ePTFE surface immobilized with Ac-$(YK)_3G_3$LDV *in vitro*, as the LDV ligand preferentially binds to integrin $\alpha_4\beta_1$ expressed on endothelial cells. In the case of one-week culture, adherent endothelial cells were maintained and proliferated on this surface. In the *in vivo* experiment, most of the surface area of the ePTFE patch immobilized with Ac-$(YK)_3G_3$LDV was covered by tunica intima-like tissue with no clot formation after 14 days of implantation on the carotid artery. Additionally, the *in vitro* adhesion of mesenchymal stem cells, known as endothelial progenitor cells, onto the ePTFE surface was specifically enhanced with the co-immobilization of integrin α_{11} ligand peptide and heparin.

Bioinert: Anti-biofouling surface

OP surfaces were successfully prepared with a smooth topology (Ra≈6 nm) and low water contact angle (≈30º). Adsorption of serum proteins was drastically suppressed by the immobilization of oligoprolines. The adsorbed amount of albumin and fibrinogen on OP surfaces was less than 10 ng/cm^2 and 40 ng/cm^2, respectively. Adhesion of fibroblasts was strongly suppressed on OP surfaces *in vitro*. After contacting whole blood for 5 minutes under the *in vitro* dynamic condition, the surface coverage by blood elements including blood cells and platelets on OP surfaces was much lower than that of the clinically available polyurethane. Adhesion of $CD62P^+$ platelets and $CD45^+$ leukocytes was strongly suppressed on OP surfaces. Furthermore, OP surfaces did not allow the adhesion even with *E coli.* and *Staphylococcus aureus* which is known to secrete prolyl aminopeptidase.

Conclusions

The immobilization of ligand peptides to specific receptors enhanced the adhesion of target cells to the material surfaces of medical devices. On the other hand, surfaces immobilized with collagen-backbone-inspired peptides, such as oligo-prolines, demonstrated good anti-biofouling properties. Thus, the immobilization of peptides can contribute to the development of blood-compatible devices with both bioactive and bioinert surfaces.

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ENGINEERING BIOMATERIALS FOR BIOFABRICATION AND 3D *IN VITRO* MODELS

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Introduction

The significant achievements in the biodesign of biomaterials have possibly to advance several research fields, including tissue engineering, biofabrication, and *in vitro* models [2]. The existing plethora of biomaterials, ranging from natural to synthetic, can be produced, functionalized and processed to become similar to natural extracellular matrix (ECM), making them ideal temporary supports for cells and tissue regeneration [2].

This lecture will present and discuss the recent progresses in the engineering of biomaterials at the 3B's Research Group (Univ. Minho, Portugal) for applications in 3D printing of complex tissues and 3D *in vitro* tissue/tumour models. The integration of biomaterials with microfluidics and 3D printing brings a myriad of advantages for manufacturing complex tissue designs and advanced dynamic culture systems that can play a pivotal role in personalized medicine, particularly in in vitro diagnostics tools and disease modeling [3]. Few examples on how biofabrication techniques are synergizing with advanced soft materials and microfluidic platforms will be provided. The main challenges, limitations of biofabrication techniques (e.g., 3D bioprinting, hot embossing, lithography, and laser ablation techniques) employed in the fabrication development of soft microfluidic devices will be also discussed. Finally, a future outlook will be presented, where either natural-based and soft biomaterials and advanced bioinks can open up new avenues for integrating more complex cellular and microbial components to better emulate tissue/organ physiology.

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METAL FLUOROPHOSPHATE BASED NANOPOWDERS FOR WOUND HEALING

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Introduction

A break in the continuity of a body tissue due to an external action is referred as wound. Wound care is a substantial healthcare concern and wound healing is a complex process that includes coagulation, inflammation, angiogenesis, NEW TISSUE DEVELOPMENT and ECM modification. Certain types of wounds like burn wounds, bed sores and diabetic wounds are always a challenge for the physicians to get them healed. The basic pathology that underlies these wounds that makes them slow to heal is their relative avascularity or impaired angiogenesis. The search for the material to increase the healing potential of the external applications, which would assist expedited healing, decrease inflammation, and prevent scar formation associated with cutaneous wounds, is ongoing for years without a breakthrough. Hence there is a need for a biocompatible non-toxic formulation which is easy and comfortable for topical application that can hasten the wound healing by inducing angiogenesis, facilitating epidermal and fibroblast migration and proliferation.

Materials and Methods

The materials used in this study are PPG 4000 (Polypropylene Glycol), Silver sulfadiazine USP, Vaseline, and nanopowder of metal-oxide-doped Fluorophosphate glasses. The ideal base for the composite was selected between PPF, 1,2-diol, 1,3-diol, PEG and PPG by their toxicity on the HEK, HDF and HUVEC cell lines. Since PPG was the least toxic, PPG nano fluorophosphate composites potency for secretion of FGF, EGF, and VEGF on corresponding cell lines was evaluated. In-vivo studies were performed on 72 albino Wistar rats, with cut and burn wounds (modified Mason Walker Burn Wounds) created and treated with experimental ointments. Wound healing progress was documented through photographs and dressing changes on various days (3, 7, 10, 14). Histopathological, histochemical (VEGF, CD31), and immunochemical (Ki67) evaluations were conducted on harvested specimens.

Results and Discussion

Histo-pathological features were assessed and tabulated for 14 factors, including epithelial ulceration, neo-vascularisation, lymphocytes, plasma cells, granulation tissue, dystrophic calcification, exudates, nerves, muscular discontinuity, inflammation in subcutis, hair follicles, adnexal structures, subcutis, and collagen synthesis. Results were recorded on the 3rd, 7th, and 14th days for both cut and burn wounds. Day 7 and 14 specimens were used for immune histochemical (IHC) evaluation of vascular endothelial growth factor (VEGF) and cluster differentiation 31 (CD31), as well as IHC fluorescence evaluation of the progressive healing factor Ki67. Magnesium demonstrated superior tolerance; with a 50% toxic limit over 3.5 mg, surpassing other substances. In scratch assays, Magnesium outperformed controls at 10 and 100 µg concentrations and 3 and 6-h intervals. PPG base showed the least toxicity among all bases tried. Evaluation of Nano powder composites revealed Magnesium as the least toxic and with increased secretion of FGF, EGF, and VEGF. In vivo evaluation highlighted the pronounced neo-vascularisation, adnexal structure formation, and hair follicle reappearance with magnesium composite treatment, emphasizing tissue regeneration. While external healing was better with controls, the experimental group exhibited sustained deeper healing, aligning with the recreation of superficial and deep layers. Histo-chemical evaluation on day 7 and day 14 showed sustained activity of CD31 and VEGF in the magnesium group, followed by the zinc group. The progressive factor Ki67 had maximum expression with magnesium composite in the histo immuno chemical evolution. Epithelial closure was comparable across all experimental groups. In cut wounds, experimental fluorophosphate ointments were on par with controls, but in burn wounds, where ischemia played a crucial role, experimental ointments excelled in histopathology, histochemistry, and immunochemistry evaluations. The enhanced healing potential of the active ingredient, attributed to Nano-sized particles of the fluorophosphate glass, was evident.

Conclusions

Overall, the study highlights the potential of metal oxide doped fluorophosphate glass in a topical formulation for wound healing. The study identifies magnesium-doped fluorophosphate glass as the most effective in promoting wound healing. Toxicity evaluation, scratch assay and CAM evaluation support the superiority of magnesium-doped fluorophosphate glass. PPG is identified as the suitable base material. In vivo evaluations on rat models further confirm the positive effects, especially in burn wounds. The topical formulation outperforms the positive and negative controls in healing burn wounds with ischemia as the key pathology. It is non-toxic, biocompatible, easy to apply and also promoting the secretion of vital growth factors (VEGF, EGF, FGF) that accelerate healing for cut and ischemic wounds. The nano-sized metal-oxide-doped fluorophosphates glass enhances healing by facilitating easy dissolution of calcium and fluorine ions. Hence we conclude that the formulation with magnesium-doped fluorophosphate glass holds promise for wound healing, warranting further research and clinical trials.

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FABRICATION AND MECHANICAL CHARACTERIZATION OF NEAR-FIELD ELECTROSPUN BIORESORBABLE VASCULAR GRAFTS MIMICKING THE ARTERIAL EXTRACELLULAR MATRIX

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Introduction

Cardiovascular disease, arteriosclerosis, is a global health problem characterized by the thickening of arterial walls restricting blood flow. One treatment option is a surgical procedure utilizing an autologous or synthetic vascular graft to bypass or replace the diseased arterial segment. Sources of autologous grafts are limited due to need for multiple grafts or lack of health tissues, and more than half of synthetic grafts fail in small diameter (> 6 mm) applications within two-years due to thrombosis and hyperplasia caused by poor reendothelialization and a mismatch in mechanical properties of the synthetic graft and the native vasculature. It was hypothesized that by mimicking the arterial extracellular matrix in bioresorbable vascular grafts via near-field electrospinning (NFES), vascular templates could be produced with mechanical properties that mimic the native vasculature more accurately than the current synthetic vascular grafts while maintaining sufficient pore sizes to allow transmural ingrowth of capillaries and the reendothelialization of the graft lumen via sprouting endothelial cells.

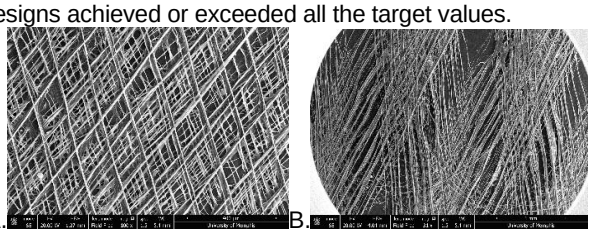
Materials and Methods

Polydioxanone vascular constructs with circumferential fiber alignment angles of 15°/75° and 30°/60° (0° representing circumferential fiber alignment) were fabricated using a custom built NFES system described in previous literature [1]. Polydioxanone was dissolved overnight in 1,1,1,3,3,3-hexafluoro-2-propanol at concentrations of 100 mg/mL. 1 mL of the polymer solution was loaded into a polypropylene syringe with a blunt, 23-gauge Luer-Lock, 2-inch stainless steel needle and mounted vertically in the NFES print head with a 1.7 mm airgap, an applied voltage of 1.6kV, and a polymer flowrate of 25 µL/h. The rotational velocity of the mandrel controlled by Q Programmer software and the translational velocity of the printhead controlled by a custom G-code were chosen to achieve a resultant fiber angle relative to the long axis of the mandrel of either 15° or 30° and a resultant polymer jet velocity of 80 mm/s at the desired angle.

Scanning electron microscopy was used to obtain images of the templates, and fiber alignment was verified using the windows 11 protractor function. Mechanical properties of the vascular constructs were measured via longitudinal and circumferential uniaxial mechanical testing, suture retention, and burst pressure evaluations, and the results were compared to literature values of the saphenous vein (SV) and internal mammary artery (IMA) mechanical properties [2]. Statistical analyses were performed using a two-sample t-test with a significance of $p < 0.05$ to determine significant differences in the two constructs.

Results and Discussion

The 15°/75° and 30°/60° fiber alignments of the vascular templates were produced as programmed (FIG. 1). In the circumferential axis testing, both templates met only the ultimate tensile stress (UTS) target value for the SV (2.61 ± 0.67 MPa) with the 15° templates having a UTS of 3.37 ± 0.21 MPa and the 30° templates having a UTS of 3.41 ± 0.38 MPa. In the longitudinal axis testing, both templates met only the IMA target value (4.3 MPa) as the 15° templates had a UTS of 4.76 ± 1.1 MPa and 30° templates had a UTS of 4.33 ± 0.47 MPa. For circumferential total percent elongation, both templates met the IMA (242%) and SV (134%) target values and surpassed the Gore-Tex® value of 70.5%, with 15° templates having a total percent elongation of 360 ± 18.9% and 30° having a total percent elongation of 276 ± 91.9%. Similarly, in the longitudinal axis testing, both templates surpassed the SV (83%) and IMA (59%) values with 15° having a percent elongation of 155 ± 19.4% and 30° having a percent elongation of 164 ± 16.7. In suture retention, the 15° template (298 gf) met both the IMA (138 gf) and SV (185 gf) values, while the 30° template (137 gf) met neither. Neither template met the SV (1599 ± 892 mmHg) or IMA (3196 ± 1264 mmHg) values for burst pressure, however, the 15° template was within the lower error for the SV with a maximum pressure of 1304 ± 109 mmHg. These results demonstrated that the 15°/75° templates were closest to mimicking the native vessel target properties as compared to the 30°; however, neither of the vascular template designs achieved or exceeded all the target values.



A. B.

FIG. 1. Representative images of fiber alignment angle verification of A. 15°/75° (magnification 31x) and B. 30°/60° (magnification 120x) templates.

Conclusions

An off-the-shelf vascular graft that reduces the risk of mechanical mismatch and thrombosis is needed to improve the patency rates of synthetic off-the-shelf grafts. Bioresorbable NFES vascular grafts propose a solution by acting as a template to enable regeneration of neointima, endothelial cell lining, as well as the arterial wall, resulting in a functioning blood vessel. Our NFES vascular templates were produced with accurate fiber alignment angles. Both 15°/75° and 30°/60° templates had appropriate circumferential and longitudinal ultimate tensile strength and percent elongation as well as suture retention strength. In summary, with future modifications NFES grafts show promise in being the next generation of small-diameter vascular grafts by mimicking native arterial properties and promoting fully functional arterial generation.

Acknowledgements

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DEVELOPMENT OF A PORCINE DECELLULARIZED EXTRACELLULAR MATRIX (dECM) BIOINK FOR 3D BIOPRINTING OF MENISCUS TISSUE ENGINEERING: FORMULATION, CHARACTERIZATION AND BIOLOGICAL EVALUATION

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Introduction
This study aims to present easily scalable, cost efficient process of dECM extraction from porcine meniscus, dedicated for bioink preparation and 3D bioprinting. The meniscus, which is cartilage-like, highly elastic, and rigid tissue, is a challenging tissue when it comes to extraction and decellularization of its ECM. Its processing poses a great difficulty and renders the methods previously developed for soft tissues useless.

Materials and Methods
To meet this challenge, a process combining homogenization, hydrolysis, supercritical CO2 (scCO2) extraction, and lyophilization was developed. This protocol allows for retaining its native compounds and biocompatibility, while offering good printability, and providing a stimulatory environment for cell proliferation and differentiation toward meniscus-like phenotype. Also, this process is economically and ecologically friendly since it doesn't require the usage of high amounts of solvents, detergents, or expensive enzymes (DNase). Nowadays, concern for the environment and the demand for sustainable resources are increasing, and the development of technologies enabling large-scale production is highly valued. The decellularization process has been meticulously studied, demonstrating a substantial reduction, however still exceeding accepted thresholds, in DNA content.

Results and Discussion
The study further explores the biocompatibility of the dECM, demonstrating no detrimental effects of remnant DNA on cell survival during extended in vitro culture, indicating excellent biocompatibility. These findings challenge the current definition of decellularization effectiveness based solely on DNA content, proposing a broader assessment of biological effects. The cells bioprinted in dECM hydrogel showed a shift in morphology, the overproduction of meniscus-specific ECM components, and expression of marker genes, suggestive of differentiation into fibrochondrocyte-like cells specific to the meniscus. Also, mechanical analysis reveals an increase in Young's modulus over culture time, indicating ongoing cellular microenvironment remodeling. For further enhancement in ASC differentiation, the study investigates the formulation of a specialized medium, composed of DMEM low glucose with 10% FBS, 2-phosphoL-ascorbic acid, dexamethasone, and TGF-β1, to enhance collagen and aggrecan production, eventually surpassing commercial chondrogenic medium results.

Conclusions
Available data indicates that the immunological safety of porcine ECM-based biomaterials seems to be unrelated to residual DNA content themselves, invalidating the need for extensive processing, which may compromise the material`s bioactive properties. In light of these findings, we proposed the utilization of the dECM containing residual DNA fragments for 3D bioprinting. The obtained dECM-based bioink was proven biocompatible, maintaining high cell viability, and increased expression and translation levels of proteins involved in chondrogenesis and the deposition of ECM components specific to the meniscus. Our research also indicates the need for additional stimulation of ASC cells differentiation, hence we propose pre-implantation *in-vitro* culturing of 3D bioprinted constructs in a medium containing 10% FBS, ascorbic acid, dexamethasone, and TGF-β1 for enhanced ASC differentiation and production of ECM.

Acknowledgements
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NOVEL CARBON-BASED MATERIALS IN EXHALED AIR METABOLOME ANALYSIS FOR CHILDHOOD ASTHMA FINGERPRINTS IDENTIFICATION

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Introduction

There is no gold standard for asthma diagnosis, which typically relies on reports of clinical symptoms. More sophisticated tests could aid in the diagnostic process. The objective of this study was to evaluate the efficacy of an innovative highly porous carbon material (HPCM) for the collection of exhaled breath and its metabolomic analysis.

Material and Methods

HPCM and Tedlar® bags were used for the collection of exhaled breath samples from 11 healthy school-aged children and 15 children with diagnosed asthma. Gas chromatography-mass spectrometry (GC-MS) was used for analysis to identify and compare volatile organic compounds (VOCs) to detect differences in exhaled breath composition between the studied groups (FIG. 1), focusing on the significance of identified chemical compounds, and to explore potential new metabolic pathways suggested by these VOCs.

Results

Both the HPCM and Tedlar® bags effectively collected exhaled breath samples from all participants. The GC-MS analysis revealed differences in the VOC profiles between the asthmatic and healthy children, with 9 repeatable and significant chemical compounds identified using both collection methods (FIG. 2). The quality of data, indicated by signal intensity, was superior in samples collected with the HPCM compared to those from Tedlar® bags.

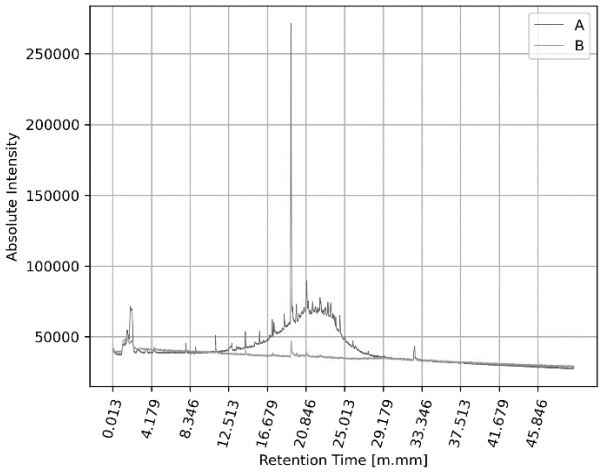

FIG. 1. Registered molecular fingerprint for healthy children B and for asthmatic children A.

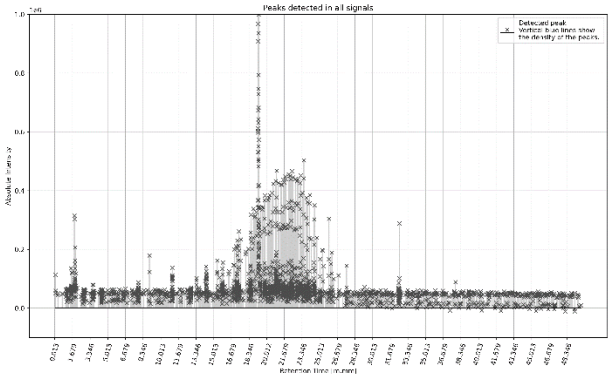

FIG. 2. The peaks indicated in the signal by the neural network recorded for asthma children.

Conclusions

The HPCM was found to be similarly effective to Tedlar® bags for the non-invasive collection of exhaled breath samples in the pediatric population. It may allow the identification of a range of VOCs and discrimination between the exhaled breath composition of healthy and asthmatic children. These results can be considered for wider application in the development of new non-invasive diagnostic tools for asthma and possibly in the search for new treatment targets.

Keywords: Pediatric Asthma, Breathomics, Highly porous Carbon Material, Volatile Organic Compounds, Non-Invasive Diagnostics.

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PHYSICAL AND CHEMICAL PROPERTIES OF SANDWICH-TYPE POLY(D, L-LACTIDE-GLYCOLIDE) COATING CONTAINING HYDROXYAPATITE AND DEXAMETHASONE ON TITANIUM ALLOY SUBSTRATE

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Introduction

Titanium alloys are widely used in medical applications, especially as implants for orthopedics. However, it is necessary to propose a surface modification method to improve their biocompatibility. The most commonly used method is anodic oxidation, which provides high corrosion resistance, but does not eliminate the presence of metal ions released into the environment [1]. A method that can limit the release of alloying element ions is the application of polymer coatings [2]. In addition, biodegradable coatings can also constitute a matrix for the release of mineral substances, such as hydroxyapatite, which can stimulate the process of bone union, as well as drugs with anti-inflammatory and antimicrobial properties.

The aim of the research was to determine the physical and chemical properties of biodegradable PLGA polymer coatings containing hydroxyapatite and the active substance on a Ti6Al7Nb alloy substrate, deposited by ultrasonic spraying.

Materials and Methods

The surface of Ti6Al7Nb alloy substrate was ground, sandblasted and anodically oxidated with the use of the electrolyte based on phosphorus and sulphuric acid at the voltage 97 V. Polymer coating based on poly(D,L-lactide-coglycolide) PLGA(85/15) was synthesized in bulk by the ring opening polymerization of glycolide and D,L-lactide at argon atmosphere. Coatings was applied by ultrasonic spray system ExactaCoat (Sono-Tek) with Impact nozzle with following parameters: ultrasound frequency 60 kHz, ultrasound power 1,5 W, speed of nozzle motion 10 mm/s and liquid's flow rate 1 cm^3/min. In the first stage, a solution of 1% PLGA in CH_2Cl_2 enriched with 20% of synthetic nanoparticle hydroxyapatite powder (<200 nm particle size) (Merck) was used for application. Coating was composed of 15 layers. Then, using a 1% solution enriched with 20% dexamethasone, 5 layers of the outer coating were applied. Samples were immersed in PBS's solution at 37ºC for 3, 6 and 9 weeks.

The scope of the research carried out included observations of surfaces using light microscopy and scanning electron microscopy, examination of surface topography using optical profilometry, examination of surface wettability, examination of adhesion of coatings to the substrate by scanning acoustic microscopy and scratch-test, examination of phase composition using XRD, electrochemical tests including EIS and pitting corrosion resistance tests, ion permeation tests from the surface, polymer molecular weight change tests, dexamethasone release kinetics, cytotoxicity tests and pro-inflammatory cytokine tests. The tests were carried out for samples in their initial state and after exposure in conditions simulating the tissue environment.

Results and Discussion

The obtained coatings were characterized by continuity, transparency and uniform particle distribution. The coatings showed good adhesion, both in the initial state and after exposure. The surface was characterized by good parameters describing corrosion resistance. Moreover, the coatings reduced the amount of ions released from the surface compared to the uncoated surface. HAp release was observed after 6 weeks of exposure. The analyzed coatings did not show cytotoxicity, but reduced pro-inflammatory cytokines.

Conclusions

Based on the obtained test results, it can be concluded that the applied PLGA coatings containing HAp and dexamethasone can improve the biocompatibility of the titanium alloy by reducing the amount of metal ions released from the implant surface. In addition, they can reduce the risk of infection through the use of the drug and stimulate the process of bone union during the release of hydroxyapatite.

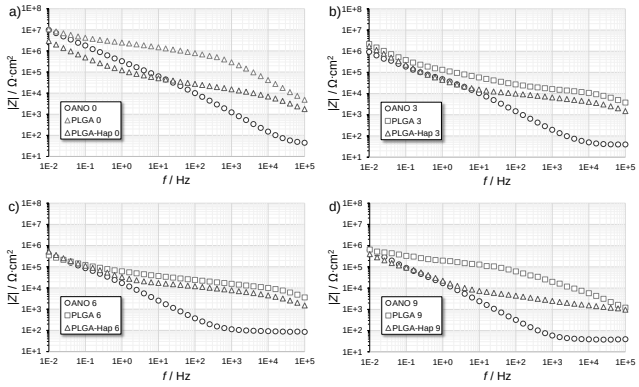

FIG. 1. Bode plots: a) in initial state, b) after 3 weeks, c) after 6 weeks, and d) after 9 weeks of exposure.

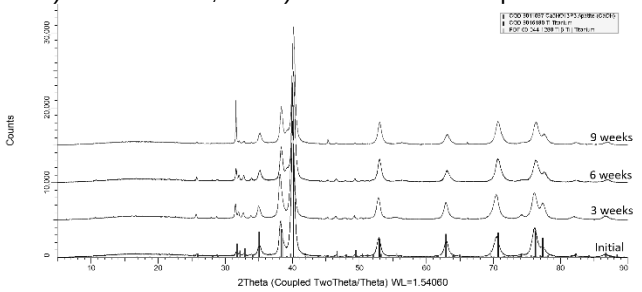

FIG. 2. X-ray diffraction patterns.

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CHARACTERISTICS OF SILICON OXIDE THIN FILMS PREPARED BY ELECTROPHORETIC DEPOSITION METHOD (EPD) FOR IMPLANTS FOR CARDIOVASCULAR APPLICATIONS

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Introduction

Years of collaborative research between physicians and engineers have advanced the modeling of cardiovascular structures and processes, increasing the ability to test and simulate pathological conditions. NiTi alloy-based implants are widely used to treat cardiovascular diseases, but their effectiveness is limited by complications such as allergic reactions and thromboembolic disease. The research underscores that the physicochemical properties of NiTi surfaces are critical to implant success, prompting a focus on developing coatings that reduce blood clotting and improve biocompatibility. This research aims to correlate the structure and physicochemical properties of silicon oxide-based coatings with their hemocompatibility [1-3]. Silicon have been used in many fields such as biomedical implants and surgical instruments mainly due to their excellent corrosion resistance and high ductility. It is also highly visible under X-rays, unlike the NiTi alloy, which is of fundamental importance when used for occluders due to their implantation method. Thus, there are indications in which the use of a biocompatible silicon oxide layer will improve the fluoroscopic visibility, limiting the release of Ni ions into the cardiovascular system and decreasing thromboembolic complications of implants made of NiTi alloy. On the ground of data found in literature, the problem of modification of biomaterials by application of hemocompatible layers of has not been completely solved. Mainly, the authors present their use due to their good optical properties [4-6]. That is why the application of the foregoing layers onto metallic biomaterials with the Electrophoretic Deposition (EPD) has been suggested in the research. The main goal of the research was presented in the FIG. 1.

Materials and Methods

Conditions were developed for depositing SiO_2 surface layers using EPD on NiTi shape memory alloy substrates. Samples underwent surface treatments, including electrolytic polishing and passivation, which are essential for shaping metal biomaterials for implants. SiO_2 layers were applied using EPD with varying deposition parameters such as time, voltage, and suspension preparation. The layers were evaluated for adhesion and ion release in artificial plasma. Corrosion resistance tests, particularly pitting corrosion, were performed on samples under simulated tissue conditions. Contact angle measurements were also conducted to assess moisture absorption, impacting implant strength and inflammation risk. In addition, the surface roughness was examined both before and after coating.

Results and Discussion

Correct surface preparation prior to coating application is crucial for adhesion and corrosion resistance.

Conclusions

The study showed that the deposition parameters of SiO_2 layer application using the EPD method has a significant effect on the coating properties. The coating, as expected, reduced ion permeation into the artificial plasma solution. Surface roughness tests showed that surface roughness increased after coating with the chosen method.

Acknowledgements

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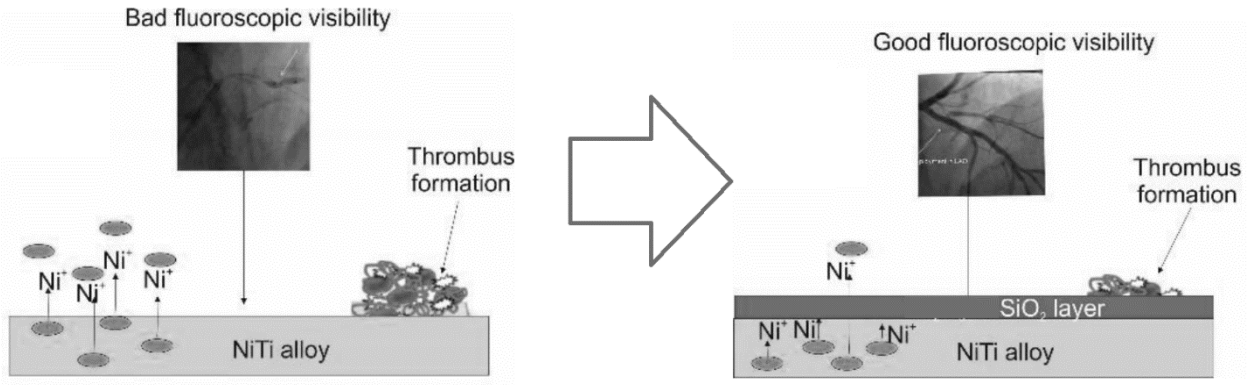

FIG. 1. The main goal of the layer is reduction of Ni ion penetration, thrombus formation and improvement of fluoroscopic visibility.

FUNCTIONALIZATION OF CARBON SURFACES FOR BIOCOMPATIBILITY: OXYGEN VERSUS AMMONIA PLASMA

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Introduction

Carbon materials, particularly graphenic ones, have received significant attention across various branches of science due to their distinct physiochemical properties and diversity in structure. These materials have been widely investigated for various applications including electronics, catalysis, energy storage, sensors and biomaterials, to mention a few. However, graphenic materials cannot be directly utilized for bioapplications in their native state because they are hydrophobic and chemically inert. Therefore, to be readily used the surface properties of these materials such as electronic and wettability needed to be modified. This can be achieved by controlled modification, introducing heteroatoms/polar groups or generating topography in the nano and microscale. The methods applied for the functionalization of the carbon materials are based on wet (strong chemical agents, *e.g.*, acids; classical way) or dry (plasma) treatment. Plasma is preferred for functionalization as it can modify the surface properties while preserving the bulk structure [1,2]. Plasma treatment is environmentally friendly, energy and time efficient and can be precisely controlled. The aim of this investigation is to explore the tunning of surface properties (electronic, wettability and biocompatibility) of graphenic material using low-temperature plasma of different gases (such as O_2, NH_3). The applied approach involves thorough characterization of the surfaces before and after plasma treatment with the use of several microscopic and spectroscopic methods. Additionally, to gain better insight into the surface's electronic properties, wettability and biocompatibility the work function measurements, water contact angle measurements and biological tests were performed (cell tests, bacterial adhesion), respectively. For molecular-level interpretation, the experimental results were corroborated with theoretical modeling (DFT).

Materials and Methods

The graphene sheet was cut into 1x1 cm coupons and then modified with plasma (O_2, NH_3). The graphenic sheets (before and after modifications) were characterised by AFM, SEM, XPS, LDI-MS, RS, TG and change in surface properties were measured using work function (Kelvin Probe) and water contact angle measurements. The experimental results of graphenic surfaces functionalization were supported by DFT molecular modelling of surface functional groups geometry, dipole moments and their effect on Fermi level and surface potential. Additionally, the biological tests for cell attachment (mouse fibroblast NIH/3T3 cell line ATCC CRL-1658) as well as Gram+ and Gram- bacterial adhesion (S. aureus and P. aeruginosa) were performed.

Results and Discussion

Microscopic images (SEM and AFM) showed that the topography of the surface remained intact after modification with plasma for even 5 mins. Results from XPS and SIMS showed the presence of nitrogen and oxygen functional groups attached to the surface after plasma treatment. The most abundant was the -OH, -CO, -COO and $-NH_2$, -NH, $-CNH_X$, $-CH_2NO$. The generated functional groups dramatically affect the physical chemical properties of the graphenic surface. The surface from hydrophobic (98°) become hydrophilic, reaching <5° for O_2 plasma and <20° for NH_3 plasma. Also, the the electronic properties have been effectively modified with the work function changing by up to 1.4 eV and 0.4 eV for O_2 and NH_3 plasma, respectively. The extent of these effects depends on the plasma parameters, primarily the applied power. The results were interpreted in terms of the redistribution of electron density due to difference in electronegativity between the surface carbon atoms and the attached nitrogen and oxygen functionalities. The induced modifications result in biological response of the graphenic surface, with modified surfaces being superior for cell adhesion in comparison to unmodified one. The cell adhesion test reveals similar behaviour regardless the plasma used, cells appeared stressed and circular before modifications but looked more relaxed and elongated on the graphenic surface after modification. The situation was quite different for bacterial adhesion. It was observed that bacterial adhesion increases after modification of the surface with O_2 plasma whereas for NH_3 plasma, bacterial adhesion does not increase and remains similar to that of unmodified graphene paper. Typical example of the results from cell adhesion tests is presented in FIG. 1, where unmodified and ammonia plasma-modified graphenic surface are directly compared. Clear differences in cell morphology and the advantage of NH_3 plasma modification can be noticed.

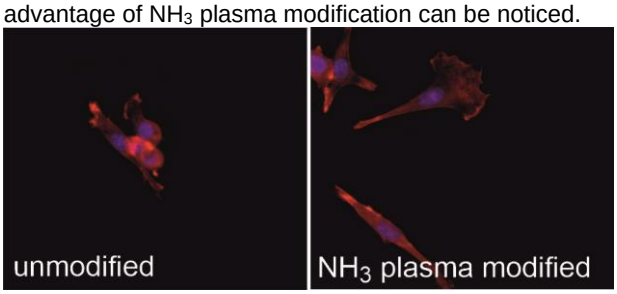

FIG. 1. The comparison of unmodified and ammonia plasma-modified graphenic surfaces illustrates the beneficial role of surface modification in enhancing fibroblast cell adhesion.

Conclusions

It is demonstrated that controlled plasma modification can be successfully used to improve the properties of carbon-based biomaterials. While the adhesion of cells can be substantially enhanced (independent on the applied plasma), bacterial attachment is not promoted (in the case of NH_3 plasma). The results are interpreted in terms of changing the electronic structure of the surfaces upon generation of functional groups, their polarity and resulting wettability.

Acknowledgements

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WHEY PROTEIN ISOLATE/POLY-γ-GLUTAMIC ACID HYDROGELS PROMOTE THE PROLIFERATION AND OSTEOGENIC DIFFERENTIATION OF PRE-OSTEOBLASTS

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Introduction

Osteo-related pathologies effect around 200 million people, globally [1]. Current gold standard treatments present limitations [2]. Therefore, there is a requirement for biomaterials in the form of scaffolds for osseous regeneration. Whey protein isolate (WPI), a dairy industry waste product, in the form of hydrogel has demonstrated potential to fulfil the role. WPI hydrogels have been loaded with hydrophobic and inorganic molecules, demonstrating the cellular proliferation, differentiation and functioning of pre-osteoblasts [3,4]. Likewise, a protein-like polymeric substance, hydrophilic poly-γ-glutamic acid (γ-PGA), has demonstrated potential in bone regeneration by aiding in the proliferation of SaOs-2 osteosarcoma cells [5]. Therefore, WPI/γ-PGA hydrogels were synthesised and analysed *in-vitro,* physio-chemically and biologically, to evaluate their potential as bone regeneration scaffolds.

Materials and Methods

WPI/γ-PGA hydrogels were synthesised by creating a 40% (w/v) WPI solution and adding γ-PGA to achieve concentrations of 0% (control) 2.5%, 5% and 10% (w/v). Once homogenised, samples were de-gassed, and gelation was heat induced at 70°C. Swelling analysis involved incubation of 1 g WPI-CBD hydrogel in to 5mL PBS for 5 days at 37°C. Raman spectroscopy was utilised to determine the incorporation of γ-PGA in the WPI hydrogel. Mechanical, compression analysis was performed with 1cmx8mm hydrogels. Cellular viability analysis was performed on an osteoblast precursor cell line MC3T3-E1s, incubated at 37°C for 3 and 14 days. SEM was performed to image cell morphology. Osteogenic differentiation, specifically Alkaline phosphatase (ALP) activity, Collagen and Calcium production, was evaluated over a 21-day period.

Results and Discussion

Raman spectroscopy demonstrated the incorporation of γ-PGA. Cell viability assays demonstrated that the hydrogels supported cell proliferation from day 3 to 14 (FIG. 1a). All γ-PGA-containing scaffold compositions promoted cell adhesion as demonstrated by dense cellular layers. γ-PGA-containing scaffolds out-performed the WPI0 control scaffolds on day 14. Significantly, osteogenic markers suggested differentiation (FIG. 1a-c). WPI-γ-PGA hydrogels generated increasing amounts of collagen throughout the experiment, and cells presented increased alkaline phosphatase activity at day 7 and increased calcium, a biomarker for matrix mineralisation, at days 14 and 21 on the γ-PGA-containing scaffolds.

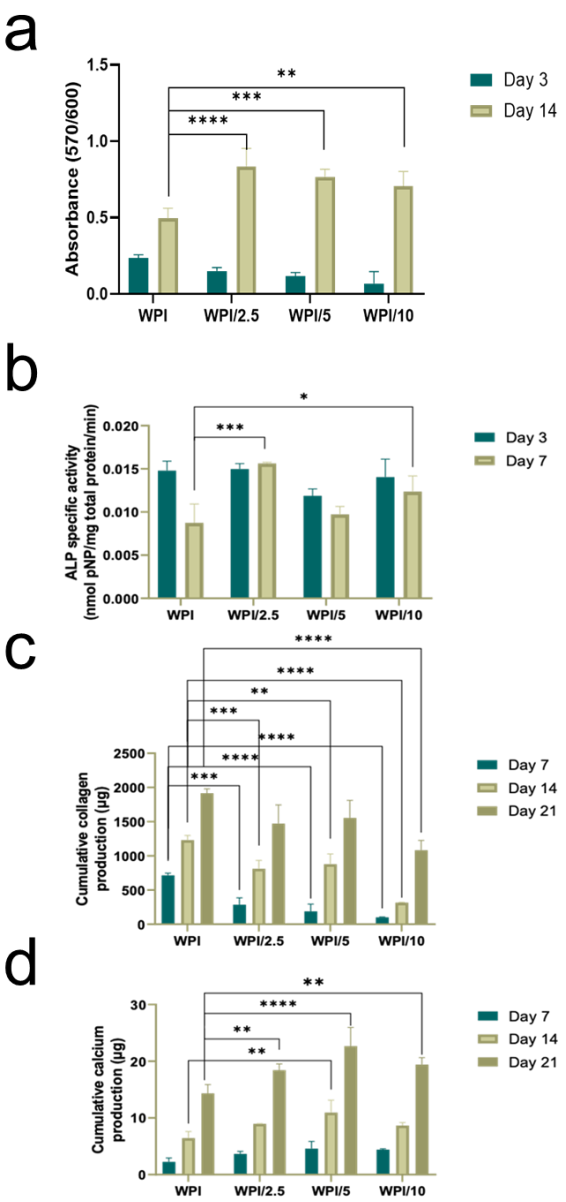

FIG. 1. The results of WPI/γ-PGA hydrogel cellular analysis. a. cellular viability analysis, b. Alkaline phosphatase activity, c. Collagen production and d. Calium production. Each bar represents the mean ± SD of n=10 (**p < 0.01, ***p < 0.001 ****p < 0.0001; compared to the WPI control).

Conclusions

WPI- γ-PGA hydrogels were successfully synthesised. All γ-PGA-containing scaffolds supported osteogenic differentiation of pre-osteoblasts. Evaluations suggest WPI/2.5 and WPI/5 are the most promising sample groups. These variables present the best cytocompatibility values. Additionally, WPI/2.5 demonstrated the highest ALP activity levels on day 7. WPI/5 showed the highest calcium production on day 21. Suggesting WPI- γ-PGA hydrogels as candidates for osseous medicine.

Acknowledgements

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SONOCHEMICAL FORMATION OF IBUPROFEN NANOPARTICLES IN WATER AND ETHANOL: TOWARDS CONTROLLED DRUG DELIVERY

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Introduction

Site-specific and controlled delivery of drugs is highly desirable in the medical field since it offers reaching the therapeutic dose at the desired site of action, whilst maintaining a minimal level of the drug. Nanosizing via ultrasound is a promising way to develop such a drug delivery system along with improving bioavailability of poorly water-soluble drugs. The so-called sonochemical synthesis involves the use of ultrasound to induce physical/chemical processes at the gas/liquid interface generated through the phenomenon of acoustic cavitation. It encompasses the formation, growth, accumulation of the molecules at the interface and subsequent collapse of cavitation bubbles, leading to the formation of nanoparticles [1]. Researchers have widely explored ultrasound-assisted synthesis of nanoparticles (NPs) of inorganic substances, however relatively little is known about the formation of NPs of bioactive substances [2-4]. In this study, we investigated the mechanism of sonochemical formation of ibuprofen NPs in water and ethanol. Ibuprofen is a chiral, non-steroidal, and anti-inflammatory drug that functions as an analgesic by inhibiting cyclooxygenase enzyme [5]. It is a widely used pharmaceutical drug for treating rheumatoid arthritis and other pains and inflammations. The common side effects associated with its peroral administration include high blood pressure, depression, etc. among others. Prescribing a reduced dosage can help minimize these effects but compromises its efficacy. Another challenge is its poor water solubility and thus its bioavailability [6]. To address these issues we propose nanosizing of ibuprofen using ultrasound thereby targeting controlled drug delivery from polymeric substrates.

Materials and Methods

Ibuprofen sodium salt procured from Merck was used for the study. Solutions of concentration varying from 30-80 mg/mL in water and ethanol were prepared. Ultrasound irradiation of the active ingredient solutions was carried out using Q500 sonicator (QSONICA) and applied the following set of parameters (power: 100 W, amplitude: 25-35%, and time: 4-8 minutes). The size and morphology of the obtained ibuprofen nanoparticles were analyzed using NTA and TEM equipped with EDX. Additionally, the structural integrity of the synthesized nanoparticles was checked using IR spectroscopy.

Results and Discussion

The results are discussed in terms of the initial precursor concentration, type of solvent, and the sonication time. From NTA results we noticed that the type of the solvent significantly influences the size of the generated ibuprofen NPs. The analysis also indicated a top-down mechanism of NP formation in water i.e., the size of the NPs in water decreased post-sonication. While in ethanol a bottom-up criteria prevailed with bigger particles forming after the ultrasound exposure.

TEM along with EDX analysis confirmed the presence of ibuprofen NPs. The size of the individual NP was found to be 40-50 nm (in water). FIG. 1 depicts the sonochemical formation of ibuprofen NPs and the hydrodynamic size and distribution of the particles from NTA. The TEM images shown in the insert confirm the size of the synthesized ibuprofen NPs. It is worth to mention that the IR spectra of ibuprofen before and after sonication remain unaltered proving the stability of the bioactive substance and thus its bioactive potential. The experimental results of ibuprofen NPs formation in water and ethanol were supported by molecular dynamics simulations revealing the diverse tendencies of ibuprofen agglomeration in both solvents.

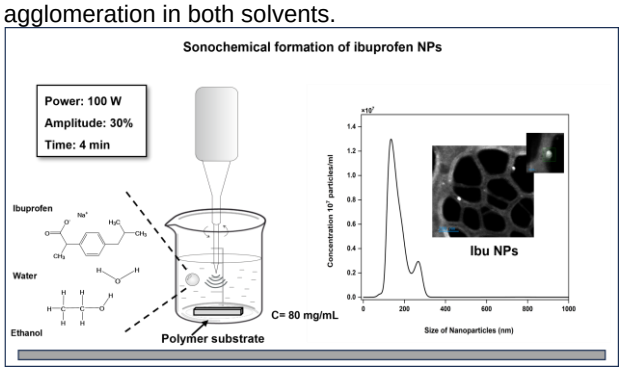

FIG. 1. Graphical scheme of the applied approach and the results: hydrodynamic diameter distribution (NTA) and corresponding TEM images of ibuprofen NPs.

Conclusions

In this study, we explored the alternate mechanisms of NP formation of ibuprofen in water and ethanol under the influence of ultrasound. It was observed that the drug depicts two entirely different criteria for forming NPs in these solvents. We also found that this approach makes it possible to tune the size of the ibuprofen NPs by adjusting the sonication parameters. The study points towards a tailor-made, controlled drug delivery system based on ultrasound-assisted embedment of ibuprofen NPs on the surface of implantable materials like polymers providing an attractive approach for effective drug administration.

Acknowledgements

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WHEY PROTEIN ISOLATE/ CALCIUM SILICATE HYDROGELS FOR BONE TISSUE ENGINEERING APPLICATIONS

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Introduction

Whey protein isolate (WPI) hydrogels are attractive biomaterials for applications in bone repair and regeneration. However, their main limitation is low mechanical strength. Therefore, to improve these properties, incorporation of ceramic phases into hydrogel matrices is currently being performed. In this study novel whey protein isolate/calcium silicate (WPI/$CaSiO_3$) hydrogel biomaterials were prepared with varying concentrations of the ceramic phase ($CaSiO_3$). The aim of this study was to investigate the effect of the introduction of $CaSiO_3$ to WPI hydrogel matrix on physicochemical, mechanical, and biological properties.

Materials and Methods

$CaSiO_3$ was added to WPI solution; 1 mL composite biomaterials were formed at 90°C for 20 minutes and autoclaved at 121°C for 2 hours. There were three sample groups with the following final concentrations: (1) 40% WPI/0% $CaSiO_3$ (control); (2) 40% WPI/2.32% $CaSiO_3$ and (3) 40% WPI/5% $CaSiO_3$. Physicochemical characterisation included swelling tests, mechanical testing and Fourier Transform Infrared (FTIR) spectroscopy. Cell biological characterisation was performed using normal human fetal osteoblasts (hFOB 1.19 cell line). Cells were seeded on biomaterials or on tissue culture polystyrene (PS) surfaces at a concentration of 1 x 10^5 cells/sample and incubated for 3 and 6 days for proliferation assays or 7 or 21 days for differentiation assays. A WST-8 assay, fluorescence microscopy and RT-qPCR analysis were performed.

Results and Discussion

FTIR results showed successful incorporation of $CaSiO_3$ into the WPI hydrogel matrix. to create composite biomaterials. 5% (w/v) $CaSiO_3$ caused greater swelling, ultimate compressive strength and strain at break. The WST-8 assay and fluorescence microscopy showed that the 40% WPI/5% $CaSiO_3$ group demonstrated superior cell proliferation in vitro, compared to the 40% WPI/0% $CaSiO_3$ group (FIG. 1). Furthermore, gene expression of osteogenic differentiation markers, namely collagen I (after 7 days), alkaline phosphatase and osteocalcin (after 21 days) were higher on the 40% WPI/5% $CaSiO_3$ group than on both the 40% WPI/0% $CaSiO_3$ group and PS (FIG. 2).

Conclusions

The addition of $CaSiO_3$ to WPI-based hydrogel biomaterials renders them more promising for bone tissue engineering applications.

Acknowledgements

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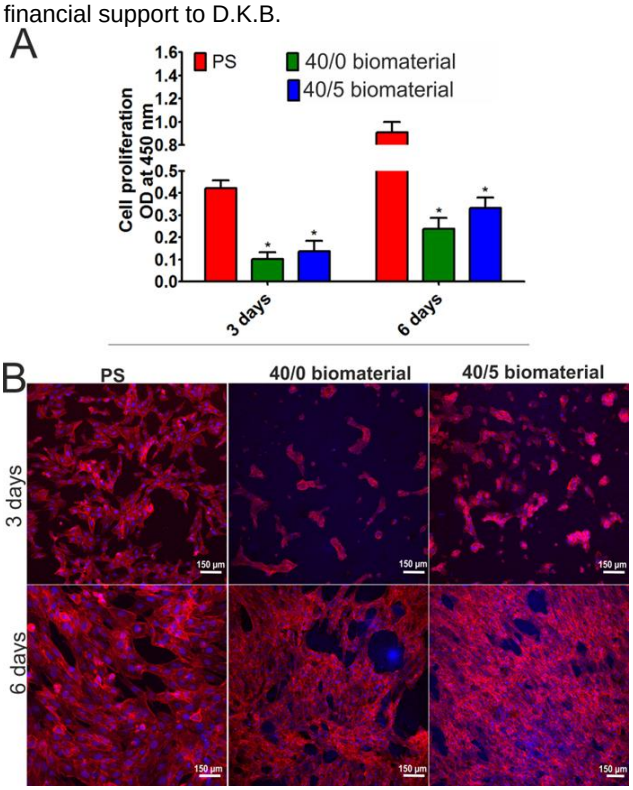

FIG. 1. Proliferation of human osteoblasts cultured on the 40% WPI/0% $CaSiO_3$ (40/0) and 40% WPI/5% $CaSiO_3$ (40/5) groups after 3 and 6 days of incubation. Metabolic activity was assessed by the WST-8 assay (A). *Significantly different results compared to control (cells cultured on polystyrene, PS), Two-Way ANOVA test, followed by Bonferroni comparison test, $p < 0.05$. No statistical differences were observed between the sample groups. Cell morphology (B) was visualized by staining cell nuclei (Hoechst 33342 dye) and actin filaments of the cytoskeleton (AlexaFluor 635 dye). Cells were observed under a confocal microscope, magnification 100x, scale bar = 150 µm.

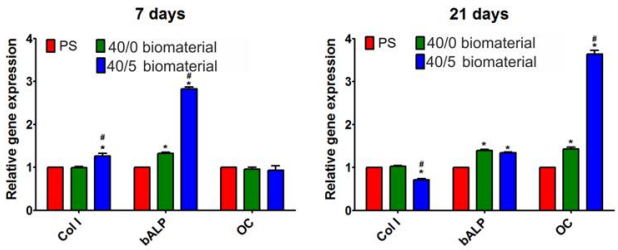

FIG. 2. Relative expression level of genes: collagen I (Col I), bone alkaline phosphatase (bALP), and osteocalcin (OC) in human osteoblasts which grew on the 40% WPI/0% $CaSiO_3$ (40/0) and 40% WPI/5% $CaSiO_3$ (40/5) groups. The data were normalized to the expression level of genes in cells maintained on polystyrene (PS). *Significantly different results compared to expression level in cells which grew on PS; #Significantly different results compared to expression level in cells which grew on the 40/0 sample group; One-Way ANOVA test followed by Tukey's multiple comparison, $p < 0.05$.

EVALUATION OF THE BIOLOGICAL SUITABILITY OF METAMATERIALS DEDICATED TO ATRAUMATIC LAPAROSCOPIC INSTRUMENTS

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Introduction

A major problem in thoracic and cardiovascular surgery is tissue traumatization leading to serious perioperative complications like blood flow disorders and life-threatening bleeding. The main focus of the project is next-generation atraumatic surgical instruments with overload protection and stress detection to control tissue pressure as the basis for trauma-free tissue grasping. The primary area of innovation is cardiovascular and thoracic surgery. The main focus of the work is new-generation surgical instruments with force overload protection and with strain detection to control tissue pressure as a basis for trauma-free tissue grasping. The project's innovation is ToolBox Technology (TBT), consisting of enhanced biocompatible sensors on gripping surfaces, metamaterials with defined reversible quasi-plastic deformation above a defined threshold for overload protection, and wear/scratch-resistant, easy-to-clean coatings that will, in the future, be applied to conventional and laparoscopic handheld and robotic surgical instruments.
Our primary area of innovation is cardiovascular surgery. State-of-the-art cardiovascular and thoracic surgery clamping and grasping instruments (CVTCGI) are not real-time controlled forceps. As a result, almost all vascular procedures run the risk of using excessive and uneven clamping/grasping forces.

Materials and Methods

Two types of materials were used as atraumatic inserts dedicated to laparoscopic instruments, metallic materials, and the polymer specimens manufactured using Fused Filament Fabrication (FFF). The mechanical behaviour of the specimens was investigated by means of compression tests. The tests were carried out using a 'Zwick/Roell Z250' at room temperature. The displacement was measured globally and locally using a digital image correlation system.
The biological suitability of materials dedicated and the evaluation of the effect of sterilization and number of cycles on cytotoxicity for the surgical tools [1] was evaluated by a correlative technique combining electron microscopy, acoustic and confocal microscopy. Under microscopy techniques, a protocol was prepared for imaging biological structures to assess cytotoxicity. Within the framework of fluorescence techniques, cytotoxicity was evaluated using molecular probes. Evaluation of the designed materials was assessed by mechanical tests followed by the observations of the tissue by histological techniques. The inflammatory cytokines analysed according to European Society of Biomaterials regulations were tested based on the Luminex xMAP technology, combining advanced fluidics, optics, and digital signal processing with proprietary microsphere. xMAP technology used fluorescence-labeled microspheres or beads, allowing for the simultaneous capture of up to 80 protein analytes or 80 genes from a single reaction. A dissection most often occurs due to local damage or rupture of the aortic wall on the inner side. Once the gate for the dissection is formed, two channels form in the vessel - one proper channel, where blood flows, and another - between the layers of the artery wall - where blood does not move. The abnormal channel can enlarge and compress the outlets of the aortic branches. Such a condition causes ischemia of the organs supplied by the artery. The evaluation of tissue traumatization with the introduction of internal pressure was designed and implemented.

Results and Discussion

On the basis of numerical analyses, the maximum possible transfer through the wall of the blood vessel was estimated.
Biological suitability studies were carried out and revealed in cytotoxicity studies which showed no negative, toxic effect in both 24 h and 48 h tests. Analysis of pro-inflammatory cytokines did not show alarming elevated levels, which prove the applicability of this type of material in medical applications. The arrangement of pores in the material structure was optimized towards atraumatic properties.
A dedicated test to analyze the degree of tissue traumatization was performed to estimate the maximum forces transmitted to the blood vessel walls. The tests were preceded by numerical analyses. The tests were performed on coronary vessels. On the basis of histological studies, delamination of the wall was found in the areas where the forces were exceeded. The determined maximum forces made it possible to design new inserts, making it impossible to exceed them and thus protecting the blood vessel tissue from crushing.

Conclusions

Metamaterials will enable:

- to increase patient safety in hand & laparoscopic surgeries by "real atraumatic" devices
- provide haptic feedback to surgeon reducing human factor
- improve surgery efficiency by reduction of surgeon workload
- imrove sustainability by eco-design of easy-to-clean, reusable & repairable surgical tools

Acknowledgements

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ACCELERATED POLYCATECHOLAMINE COATING OF COLLAGEN-SEALED VASCULAR PROSTHESES - COMPARISON OF THREE METHODS

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Introduction

Collagen-sealed polyester prostheses, commonly used in reconstructive vascular surgery, may undergo early or late infections despite perioperative antibiotic prophylaxis, which still remains the Achilles' heel of modern vascular surgery. Although the infections occur in up to 5 % of patients after reconstructions, the mortality in this group is estimated to be between 25-88% [1] and major amputations between 40-70% [2]. Rifampicin- and silver-bounded grafts, used as far, show unsatisfactory efficacy in solving this problem. Polycatecholamine coatings deposited on the grafts in order to further bind antibiotics are potentially helpful. However, accelerators of polycatecholamine polymerization may independently alter the properties of matrix, reducing its final biocompatibility and efficiency.

Materials and Methods

Polycatecholamine (poly(L-DOPA)) was deposited on collagen-sealed knitted polyester vascular prostheses via in situ polymerization in mild alkaline buffer [3] using composition of seawater-inspired ions, sodium periodate or Cu/H_2O_2 mixture as accelerators. Polycatecholamine polymerization was monitored by UV-Vis spectra analysis. The graft structure and properties were studied via FTIR, SEM, FIB-SEM and compression measurement whereas coating stability was tested in buffers, SBF (simulated body fluid) and human blood. Gentamicin was immobilized on the graft surface by catechol moiety-binding mechanism during soaking in drug solution whereas drug release profile and parameters were evaluated after prosthesis incubation in PBS. Reaction with whole human blood allowed to evaluate clot forming ability of the grafts and blood hemolysis. Antibacterial activity and bacterial adhesion was evaluated on *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity was measured with ABTS and DPPH method.

Results and Discussion

Prostheses were uniformly coated by polycatecholamine using all tested accelerators. The adhesion between poly(L-DOPA) and collagen sealant was even and tight, confirmed also by chemical interactions between the coating and collagen (by FTIR examination), although the coating layer was thicker when sodium periodate or Cu/H_2O_2 mixture were used. Antibacterial activity of all coatings was quite comparable; however, sodium periodate-accelerated coatings were less effective against *S. aureus* bacteria.

Sodium periodate-accelerated coated grafts showed the highest drug immobilization yield (over 2 fold higher than for other grafts) and mechanical strength. They also released gentamicin with the highest rate (nearly 2 fold more efficient than other grafts) but the coatings were relatively unstable in SBF.

Cu/H_2O_2-accelerated coating showed the highest antioxidant activity but also the highest blood hemolysis (~30%), notable reduction of hemostatic activity and the lowest mechanical strength and coating stability.

Seawater ions-accelerated poly(L-DOPA) coating exhibited the highest coating stability in buffers and SBF, the lowest hemolysis rate, satisfactory mechanical stability and antibacterial and antioxidant activity.

Conclusions

The results obtained in this study lead to the conclusion that the appropriate selection of accelerator of polycatecholamine coating formation is very important for medical devices. It should obviously be optimized based on careful analysis of a wide range of biological properties for each device individually. This may lead to the synthesis of the medical device of optimal properties and biological safety.

Moreover, this study points into a fact that seawater-inspired ions mixture deserves attention in design of functionalized medical devices as a novel accelerator of polycatecholamine coatings formation. This accelerator seems to be relatively safe and effective in particular medical applications

Acknowledgements

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CHEMICALLY-INDUCED JAMMING AND 3D BIOPRINTING OF GRANULAR HYDROGELS

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Introduction

The emergence of 3D Bioprinting arises due to its capacity to provide high flexibility and precise control over the final construct characteristics and composition [1]. For a successful 3D printing outcome, design and fabrication of smart inks are preferred. Granular hydrogels have recently arisen as promising bioinks. Typically made of densely packed micro-sized particles they are characterized by interparticle porosity, convenient for cell proliferation, differentiation, and migration. Their unique extrudability is given by adequate rheological properties: yield-stress, shear-thinning, and self-healing behavior [2]. The modularity and porosity make them both cell-friendly and tunable. Dynamic crosslinking allows the development of stimuli-responsive hydrogels with intrinsic reversibility triggered by tunable physical and chemical conditions [3].

Materials and Methods

This study aims to design a granular hydrogel system made of jammed disulfide crosslinked pNiPAM microgels whose printability can be tuned, not only by the weight fraction of particles but also by their in-situ swelling resulting from the selective cleavage of the disulfide crosslinker. The hydrogel inks were characterized by a combination of dynamic light scattering, rheology, and cryo-electron microscopy. For practical use for tissue engineering purposes it is critical that the scaffolds remain dimensionally stable, i.e. doesn't dissolve or deform, in cell culture conditions (physiological conditions) at least during the time frame needed for the cells proliferate all over. Therefore, using the created thiol units, inter-particle disulfide crosslinks were formed back after immersion of the printed scaffolds in a sodium periodate solution to obtain dimensionally stable scaffolds immersed in an aqueous medium (FIG. 1A).

Results and Discussion

The addition of tris-(2-carboxyethyl)phosphine hydrochloride (TCEP), a reductant, effectively led to swelling of the microgel particles due to a cleavage of the disulfide bonds (FIG. 1B). This could efficiently increase the yield-stress values of the microgel dispersions prepared at a fixed weight concentration of particles. Consequently, jammed microgel dispersions that initially showed poor shape fidelity after 3D printing, were turned into stable 3D printed constructs (FIG. 1B).

The printed scaffold was crosslinked by immersion in sodium periodate. While, without annealing, the scaffold spontaneously degrades as the microgels get dispersed and diluted in cell culture/physiological/1x-PBS medium, after annealing, the scaffolds remain stable over a prolonged period that extends at least up to 14 days in medium.

Conclusions

We successfully jammed disulfide crosslinked pNiPAM granular hydrogel systems which printability was tuned, by the weight fraction of particles and by the extent of swelling of the particles. The use of reversible covalent bonds allowed particle swelling triggered by the addition of TCEP, and stability of the printed construct by the immersion in a sodium periodate solution.

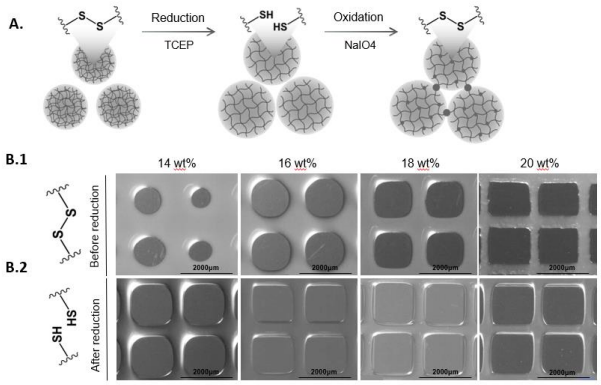

FIG. 1A. The microgels are jammed by inducing their swelling through chemical reduction of the disulfide crosslinks with TCEP and further 3D printed. The printed scaffold is cured by the re-formation of inter-particle disulfide bonds upon immersion in a NaIO4 solution after the printing process.
FIG. 1B. 3D extrusion printing of the microgels dispersion. The microgels are first dispersed in water at weight fractions ranging from 14.0 wt.% to 20 wt.%. Before (B.1) or after (B.2) reduction they are 3D printed into 8-layer square mesh scaffolds.

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SUITABILITY OF HEAT-TREATED Co-Cr-Mo ALLOY INTENDED FOR APPLICATIONS IN CARDIOLOGY

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Introduction

Cobalt alloys play a crucial role in modern medicine due to their exceptional properties. These alloys, particularly cobalt-chromium, are known for their biocompatibility, corrosion resistance, and mechanical strength [1]. The unique combination of durability and stability makes cobalt alloys an ideal choice for long-term medical applications. Despite some concerns about potential health risks, extensive studies have shown that the benefits of using cobalt alloys in medical devices significantly outweigh the risks [2]. They are widely used in medical implants such as hip and knee replacements, dental implants, and stents [3].
The following study evaluates the metallurgical quality of a heat-treated cobalt alloy depending on the heat treatment parameters adopted, i.e. annealing time, temperature, and the cooling medium used.

Materials and Methods

The test material consisted of samples of the wrought Co-Cr-Mo alloy in the form of a rod with a diameter of 5 mm. The chemical composition of the tested alloy met the requirements contained in ISO 5832-12 (“Implants for surgery - Metallic materials Part 12: Wrought cobalt-chromium-molybdenum alloy”).
The work included a heat treatment (i.e. annealing) by placing the samples in the furnace chamber and then gradually heating the material to the given temperature. When the material has reached the desired temperature was maintained for 10 minutes. Then, the cooling process was carried out in three media, i.e.: air (P), oil (O), and water (W). The temperature in the mentioned process varied in the range from 800°C to 1200°C in steps of 100°C. The total time of the applied heat treatment varied from 30 min. up to 150 min. in steps of 30 minutes.
The assessment of non-metallic inclusions was based on the recommendations of the ASTM E45-05 using a Leica DMi8 optical microscope equipped with Application Suite X software. Observations were carried out at magnifications ranging from 50-500x. classified into four groups: A (sulfides), B (clays), C (silicates) and D (oxides globular).
An etching process was performed to reveal the microstructure. Microstructure tests were carried out using differential interference contrast under magnification 50x and 200x.
The mechanical properties were determined based on hardness measurements. The measurements were performed on Dura Scan Struers universal hardness tester at the temperature of 25°C using Vickers method. The value of the applied load was equal to 1000 g.

Results and Discussion

As a result of the metallurgical quality analysis, it was found that the number of non-metallic inclusions was within the defined range provided in the standard. In order to more precisely assess the quality of the tested material, quantitative measurements, determining the average diameters of non-metallic inclusions were performed. The largest inclusion diameters were observed for samples that were cooled in oil, regardless of annealing temperature and time. The smallest average diameter of non-metallic inclusions was observed for the samples annealed at 900°C for 150 min. and cooled in air. For a temperature of 800°C, 900°C and 1200°C (annealing time 30 min.) a decreasing average value of inclusion diameter was observed, regardless of the cooling medium used. For the samples annealed for 90 minutes, the smallest differences in the average inclusion size were observed. In the case of samples that were subjected to annealing for 120 minutes, a decrease in the value of the average inclusion diameter was observed for the samples cooled in air.
As a result of the microscopic observations, it was found that the tested alloy has a single-phase austenitic structure. Microstructure analysis showed that an increase in grain size was observed with an increase in the annealing temperature. Moreover, the analysis shows that an increase in grain size results in a decrease in mechanical properties. It was noticed that the structures of samples cooled in oil were characterized by larger grains compared to samples cooled in air and water.
The hardness tests showed that as the temperature increases, the mechanical properties decrease, regardless of the cooling medium used. As the annealing time increases, the hardness value decreases for samples cooled in air and oil. In the case of samples annealed for 150 min, regardless of the cooling medium, a decrease in hardness was observed.

Conclusions

Based on the research conducted, the following conclusions were formulated:
1. Tested cobalt alloy in terms of chemical composition, microstructure, and mechanical properties meet the recommendations of ISO 5832-12.
2. The use of heat treatment (annealing) affects the morphology of the obtained microstructure, i.e. an increase in the annealing temperature causes an increase in the grain size.
3. The heat treatment used affects the mechanical properties, i.e. as the temperature increases, the strength properties decrease.
4. Increasing the heat treatment time causes grain growth, but does not significantly affect the strength properties.
5. The highest strength properties were observed for samples cooled in oil.
6. Cooling intensity (medium used - air, oil, water) does not significantly affect the change in strength properties.

Acknowledgements

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ENHANCED TENDON REGENERATION THROUGH BIOMECHANICAL APPROACHES: ADDITIVE MANUFACTURING AND MECHANICAL STIMULATION

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Introduction

Tendons are vital fibrous connective tissues that anchor muscles to bones and play a crucial role in transferring forces from muscle contractions to the skeletal structure [1]. Tendons, despite being incredibly strong and resilient, can still be vulnerable to injuries caused by overuse, trauma, or age-related degeneration. Tendon regeneration is a challenging process that necessitates innovative strategies [2]. Our research introduces a novel approach to tendon regeneration by combining additive manufacturing techniques, specifically Melt Electrowriting (MEW), with a custom-designed mechanical stimulator constructed using 3D printing technology. This unique integration of technologies offers a promising solution to the complexities of tendon regeneration, eliciting keen interest from the scientific community.

Materials and Methods

The fabrication of the wavy scaffolds involved using Melt Electrowriting (MEW) to produce Polycaprolactone (PCL) wavy scaffolds with high resolution. Mechanical performance of the scaffolds was assessed through tensile testing to determine Young's modulus and ultimate tensile strength. Human tenocyte cell culture studies were conducted on the scaffolds. The culture period was divided into two phases: phase I (pre-static culture) involved 28 days of cultivation to ensure uniform cell growth. In contrast, phase II (post-static culture) entailed dynamic and static groups and a 10-day culture period. The dynamic group scaffolds underwent mechanical stimulation using our custom-made open-source 3D-printed mechanical stimulator. We applied 10% strain for one hour, followed by one hour of rest, for 36000 cycles to mimic conditions relevant to tendon regeneration. Live/dead assays and Alamar blue assays were employed to evaluate cell viability and metabolic activity. Additionally, immunofluorescence staining assessed tendon-specific cellular markers (Tenomodulin and Scleraxis) and extracellular matrix (ECM) protein (Collagen I).

Results and Discussion

The wavy scaffolds were successfully fabricated using the MEW technique. The scaffolds demonstrated a toe region with an average of 7.8 ± 2.5% strain rate and exhibited elastic behaviour akin to natural tendons. The scaffolds displayed an average Young's modulus of 4.99 ± 2.1 MPa. Biocompatibility assessments revealed consistently high cell viability, exceeding 93% during static culture. In response to mechanical stimulation, the dynamic group manifested significantly higher cell viability (94% vs. 85%) and metabolic activity (72.5% vs. 45%) than the static group. Immunofluorescence analysis demonstrated increased cell alignment in the stretching direction and augmented expression of Actin, Collagen type I in the dynamic group.

Our study demonstrates the effectiveness of the wavy scaffolds fabricated using the MEW technique in mimicking the elastic behaviour of natural tendons. The biocompatibility assessments revealed consistently high cell viability during static culture and in response to mechanical stimulation. The significantly higher cell viability and metabolic activity in the dynamic group than in the static group underscore the importance of the dynamic mechanical environment in promoting favourable cell behaviour. Furthermore, the immunofluorescence analysis displayed increased cell alignment in the stretching direction and augmented expression of Actin, Collagen type I, Tenomodulin, and Scleraxis in the dynamic group, highlighting the positive impact of the dynamic mechanical environment on cell behaviour and gene expression, underscoring the potential of wavy scaffolds in facilitating tissue regeneration and repair. The study presents also the applicability of our cost-effective, open-source mechanical stimulator device designed for uniaxial tensile stimulation of delicate scaffolds.

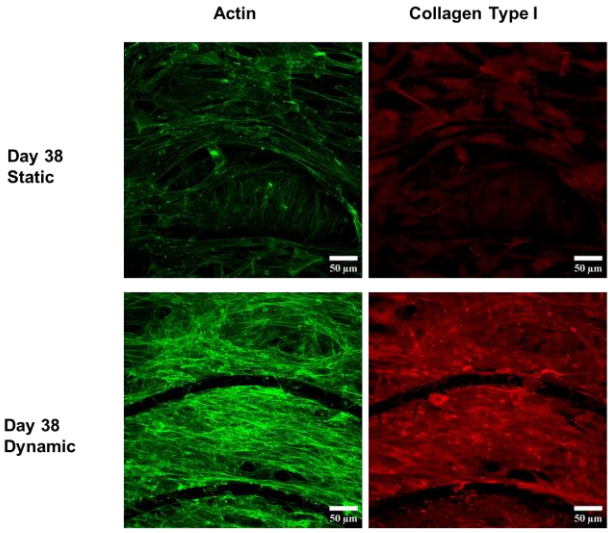

Figure 1: Immunofluorescence analysis of tenocytes grown on MEW scaffolds

Conclusions

Proposed engineered MEW scaffolds, undergoing mechanical stimulation, provided a conducive environment for tenocyte culture, showing potential for future more advanced tendon regeneration. Our findings underscore the crucial role of mechanical stimuli in driving tenocyte behaviour and ECM production. The practicality and affordability of our device make it a promising tool for future research and clinical applications.

Acknowledgements

This work was financially supported by the National Science Centre and The Polish National Agency for Academic Exchange NAWA Polish returns grant.

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BIOINK DESIGNING: BALANCING PRINTABILITY, DEGRADABILITY AND BIO-COMPATIBILITY

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Introduction

The emergence of bio-fabrication techniques like 3D bio-printing (3DBP) has widened the possibility for the regeneration of tissues. It allows to obtain scaffolds with high precision, shape versatility, and patient-specific designs [1]. A printable material comprising biomaterials, cells, and biochemical cues used to obtain stable and biocompatible scaffolds is called bioink [2]. Although multiple polymeric bioinks are available for tissue engineering (TE) applications, the portfolio of well-printable materials is still limited [3]. The optimisation of high-quality bioink is challenging, time-consuming, and demands multi-disciplinary knowledge. This difficulty could be resolved by a systematic approach using rheological assessment which enables drawing a correlation between bioink and printability aspects.
Aim: Designing a bioink based on polymers like sodium alginate (Alg), carboxy-methyl cellulose (CMC), and methacrylated gelatin (GelMA) and providing guidance for the development of well-printable bioinks

Materials and Methods

To obtain the printable bioink, compounds were mixed at different ratios and the link between rheology and 3D-printing was studied with a focus on detection of the optimized conditions for printability, stability, and biocompatibility of 3D-printed specimens.
Rheological tests of different ink compositions were performed, including flow sweep, frequency sweep, amplitude sweep, thixotropic test, temperature ramp and time sweep of dually-crosslinked inks Photo-initiator was added to formula before UV curing, followed by $CaCl_2$ crosslinking of samples. Stability of printed samples in media (with fetal bovine serum and antibiotics) was checked. Bio-printing of optimized inks with encapsulated primary fibroblasts was performed followed by live-dead assay and alamar blue assay to assess cell viability and proliferation.

Results and Discussion

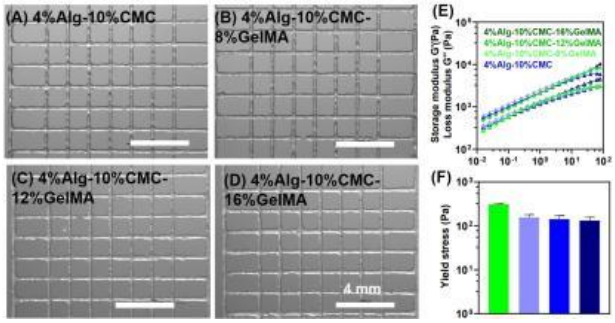

FIG. 1. Correlation between rheology and printability of 4%Alg-10%CMC-GelMA: (A-D) stereo-images of 3D printed scaffolds, (E) yield stress values, and frequency sweep (F).

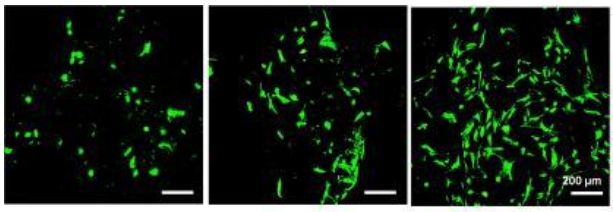

FIG. 2. Live-dead images of 3D printed scaffolds cultured for 7days.

Ink composed of 4%Alg-10%CMCand 8, 12 or 16% of GelMA was tested. Flow sweep, frequency sweep (FIG. 1E) and the thixotropic test proved shear-thinning, viscoelastic solid and self-healing nature of ink, respectively. Amplitude sweep (FIG. 1F) showed a positive correlation between yield stress, ink with high GelMA content and extrusion pressure of printing. 3D printed scaffold depicted stability for 21 days and culturing of 3DBP samples proved its cell endorsement capabilities. The cell viability differed with respect to different concentrations of GelMA and the results are depicted in FIG. 2. The live-dead assay results proved that 4%Alg-10%CMC-16%GelMA 3D bioprinted scaffolds possessed relatively higher cell proliferation capacity over 4%Alg-10%CMC-12%GelMA and 4%Alg-10%CMC-8%GelMA. However, this ink required the lowest pressure for the extrusion.

Conclusions

Characterization of rheological properties of the tested materials gave a strong indication of the shape fidelity, stability, and printability of the developed ink. Rheological assessment can be used as a powerful tool in the development of optimal printable materials with apt scaffold stability and cell viability in 3D bioprinted constructs. In further research the printed constructs are intended to be used in interface TE such as musculoskeletal interfaces.

Acknowledgements

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EVALUATION OF THE PHYSICOCHEMICAL PROPERTIES OF PMMA-BASED SPHERICAL ALUMINOSILICATES

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Introduction

Current health statistics have unequivocally shown that as the aging population grows older, there is an increasing rate of bone fractures due to weakened bone structure, particularly the incidence of osteoporosis. In the case of injuries that cause irreversible damage to joints (such as the hip or knee), an endoprosthesis is required to replace the damaged bone fragment. These types of implants require, in the case of low-density (osteoporotic) bone, sometimes the use of cement, which will provide additional reinforcement of the implant-bone connection and thus increase the patient's safety during use of the implant. Bone cement is also used as a filler for bone defects. Currently, the most commonly used materials are polymeric acrylic bone cements generally consisting of poly(methyl) methacrylate (PMMA) or calcium phosphate bone cements. Current bone cements do not fully provide these requirements, particularly those related to mechanical properties. Current work to improve this feature of bone cement is directed toward modifying the chemical and phase composition by introducing ceramic structures with similar properties to bone. One such solution is spherical aluminosilicates [1-5].

Materials and Methods

The modification process was divided into three stages. First, the aluminosilicates were etched in a fresh hot Piranha solution that had been prepared with H_2SO_4 and 30% H_2O_2 in a 3: 1 volume ratio. The acid was mixed in a beaker with a flat bottom on a magnetic stirrer (time 10 min; speed 350 rpm). After 10 min, microspheres were added and treated with Piranha solution for 30 min (speed, 350 rpm). After 30 minutes the Piranha solution was decanted into a separate beaker. To remove residual acid, the powders were filtered (4 x 1000 cm^3) with deionized water under reduced pressure using a water pump. The aluminosilicates were then silanized. The process involved soaking the aluminosilicates in a solution with Si_3N_4+NaOH (10M) for 24 hours, then the sample was placed in an ultrasonic scrubber for 10 minutes. After the time had elapsed, the powder were filtered (4 x 1000 cm^3) with deionized water under reduced pressure using a water pump. The prepared powder was dried at 80°C for 24 hours. The final stage was based on making samples with 20%, 30% and 40% aluminosilicates in a PMMA matrix.

Results and Discussion

Comparing a commercial PMMA-based bone cement and the proposed modification based on spherical aluminosilicates, there was no significant difference in the polymerization process. The relative polymerization time was about 90s. Between 5 and 6 min of polymerization, the largest temperature jump was shown. Mechanical tests showed similar values to zirconium oxide-reinforced composites. Wettability, porosity and surface (SEM-EDS) studies were performed.

Conclusions

The use of spherical aluminosilicates as a filler, will allow to obtain a composite material with significantly lower density but also with higher stiffness, stability, which will translate into a reduction in the proportion of polymer matrix in bone cement. The material used requires further research.

Acknowledgements

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POROUS IRON-BASED MATERIALS FOR USE AS CARDIOVASCULAR IMPLANTS - BIOLOGICAL AND CHEMICAL PROPERTIES

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Introduction

For years, research on biodegradable materials for implants has been dominated by polymers, because of their predictable degradation processes, ease of design, and high biocompatibility. However, there remains gap in demand for a fully biodegradable material with strength and flexibility comparable to permanent metal implants [1-2]. This is particularly critical in cardiovascular surgery, where the thickness and flexibility of stent struts are essential, limiting the use of polymers. Biodegradable metals, which corrode safely within the body over time, present a promising solution [3]. This study focuses on iron-based materials, which offer the best mechanical properties and high biocompatibility among known biodegradable metals [4-5].

Materials and Methods

The 3D iron-based scaffolds were obtained by sintering replica method, where polymer foam template was impregnated with the suspension of pure iron or mixture iron and different metal (like copper, magnesium, zinc or manganese). After that, the sample was placed in a tube furnace where in an oxygen-free atmosphere was heated until the metal melted and the template was removed.
The obtained samples were analysed by SEM, X-ray diffraction (XRD), X-ray spectrometry (EDX), and Raman spectroscopy. Corrosion tests were carried out using electrochemical and immersion methods in Hank's solution.
Biologically, cytotoxicity tests were performed (indirect test on three cell lines: mouse L929 fibroblasts, human aortic smooth muscle cells (HAMSC), and human umbilical vein endothelial cells (HUVEC).

Results and Discussion

Results were obtained enabling comparison of the influence of the template used on the final composition and properties of the sample by using two types of sponges: melamine and polyurethane. Scaffolds, created based on melamine sponge, had their carbon content increased by several percentage points. This difference was later manifested in subsequent studies of different corrosion rates and effects on cells in biological tests.
Studies comparing the influence of metallic admixtures on cytotoxicity showed significant changes compared to the cytotoxicity of pure iron. In particular, the addition of copper and manganese in appropriate percentages reduced cytotoxicity and increased cell survival. In corrosion studies, the addition of metals significantly accelerated corrosion in every case.

Conclusions

This work summarizes several years of work on materials for cheese and vessel applications made of iron by sintering. It presents the results from biological and chemical perspectives while highlighting these materials' advantages and disadvantages and the challenges they pose to researchers. Despite its many promising features for cardiovascular applications, iron still requires countless research and modification to be used as a full-fledged implant. The results presented in this presentation prove that many possible research paths should be continued.

Acknowledgements

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NANOARCHITECTURE BASED ORTHOPAEDIC IMPLANTS USING HIGH PRESSURE, ULTRASONIC AND MICROWAVE TECHNOLOGY – TOWARDS CLINICAL TESTS

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Introduction

The urgent need to address challenging bone defects resulting from various causes, including trauma, tumour resection, and joint replacements, has spurred extensive research efforts worldwide. To facilitate optimal bone regeneration and biodegradability of bone grafts and implants, the development of biomimetic nano-materials mirroring human tissue nanostructures is imperative. This study focuses on the fabrication of nanoarchitecture-based orthopaedic implants, utilizing nano-structured hydroxyapatite engineered with precise control over size and composition to emulate natural bone tissue properties.

Materials and Methods

Incorporating cutting-edge "high energy density technologies": microwave, high-pressure, and ultrasonic, we have devised innovative synthesis approaches for nanomaterials, ensuring superior quality and precision of nanostructure control. Our methodologies include Microwave Hydrothermal Synthesis (MHS) reactors for biomimetic hydroxyapatite - GoHAP production, Sono-Nano-Coating (SNC) technology for enhancing implant surfaces by means of a GoHAP layer, and production of biodegradable GoMEMBRANE designed for scaffold guided bone regeneration. High pressure techniques for permitted developing composite implants with high nano-ceramic contents. Thorough biocompatibility assessments conforming to ISO10993 standards have been conducted for GoHAP and GoMEMBRANE. They are produced according to ISO 13485 norm for medical devices.

Results and Discussion

We developed the technology to deliver six distinct sizes of GoHAP nanoparticles, each optimized for specific functionalities (FIG. 1). The smallest ones exhibit a structural likeness to cortical bone validated through X-ray diffraction analysis. Noteworthy results include the elevated calcium ion release rates of smaller nanoparticles, suggesting accelerated bone healing potential. Successful veterinary trials using GoHAP as a bone graft material have underscored its efficacy. The GoHAP membranes were successfully applied in splint bone reconstruction in horses.
GoHAP and GoMEMBRANE are ready for clinical tests.
Furthermore, our investigations explore the versatility of SNC-coatings for antibiotic absorption and advanced multilayer coating strategies to enhance implant performance. Layers differing in the size of nanoparticles or GoHAP or nano ZnO layered on top of each other were produced. Composites with GoHAP content in the range 25%-80% by volume display mechanical properties close to that of bone tissue. Further, injectable pastes have been made with a contend of up to 45% of GoHAP. Both technologies are awaiting further preclinical testing.

Conclusions

The high energy density technologies: high pressures, microwaves and ultrasounds open the way to nano architectonics in production of bone graft materials. These technologies are suitable for producing the entire range of implants required by orthopaedic surgeons. Biomimetic GoHAP nanoparticles were produced, using the Microwave Hydrothermal Synthesis (MHS), with precisely regulated size. Biodegradable GoMEMBRANES were produced using the Sono-Nano-Coating technology, coated with GoHAP, as well as nano ZnO. GoHAP and GoMEMBRANE are ready for clinical tests. Further research should include clinical studies of GoHAP and GoMEMBRANE, as well as pre-clinical tests for composites and injectable pastes.

Acknowledgements

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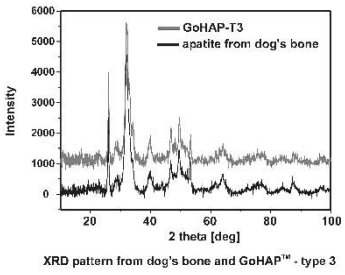
Name	Particle size calculated from SSA BET [d±σ nm]	Specific Surface Area (BET) [a,±σ m²/g]	Density [ρ±σ g/cm³]	Ca/P [Ca/P±σ]
GoHAP Type 1	9 ± 1	225 ± 20	2.82 ± 0.04	1.61 ± 0.04
GoHAP Type 2	13 ± 2	190 ± 18	2.84 ± 0.04	1.61 ± 0.04
GoHAP Type 3	16 ± 3	140 ± 15	2.86 ± 0.04	1.61 ± 0.04
GoHAP Type 4	22 ± 3	90 ± 10	2.92 ± 0.04	1.61 ± 0.04
GoHAP Type 5	32 ± 3	64 ± 7	2.97 ± 0.04	1.61± 0.04
GoHAP Type 6	42 ± 4	49 ± 5	2.98 ± 0.04	1.61 ± 0.04



XRD pattern from dog's bone and GoHAP™ - type 3

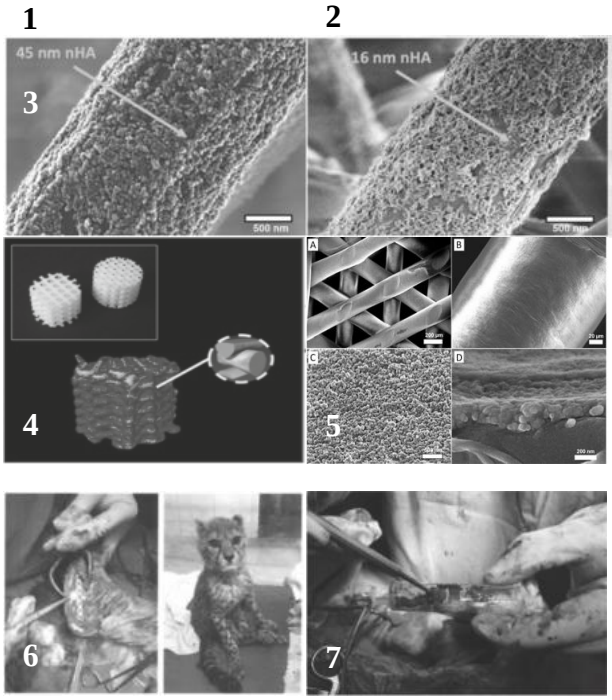


FIG. 1. Table of GoHAP particles.
FIG. 2. XRD patterns of GoHAP and dog's bone.
FIG. 3. Sonocoated GoMEMBRANE fibres.
FIG. 4 and 5. SNC coated 3D PCL scaffold.
FIG. 6. Chetachs from Warsaw ZOO operated using GoHAP.
FIG. 7. Application of GoHAP in a Dog's fractured paw operation.

FERRITE MAGNETIC NANOPARTICLES (Fe_xO_y) IN BIOMEDICAL SCIENCE: BREAKTHROUGHS IN PROTEIN FRACTIONATION AND ISOLATION

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Introduction

Iron nanoparticles (FeNPs) represent a fascinating and increasingly significant field of research in science and technology. Their unique magnetic properties, combined with their low production costs, make them highly attractive for various applications in medicine, bioengineering, and engineering. The interest in these nanoparticles is further heightened by the fact that they can be synthesized through various methods, including co-precipitation, hydrothermal, thermal decomposition, etc. techniques. These methods allow for the optimization of conditions to obtain nanoparticles with desired characteristics such as shape, size, and polydispersity [1]. One of the research directions for FeNPs includes their use as adsorbents in proteomics, particularly for preparative proteins isolation [2]. However, for biomedical applications, surface modification of the nanoparticles is necessary to enhance their biocompatibility, stability, and appropriate functionality [1,3]. This is especially crucial for materials used in analytical applications. For protein isolation, these materials need to possess appropriate functional groups on their surface, bind proteins reversibly, and remain stable over a wide pH range as well as during repeated use. Nanoparticles that meet these requirements are of the core-shell type. Formation of the shell on the surface of the magnetic core can be achieved through controlled deposition of silica layers using the Stöber process. Whereas, functional groups on the surface are introduced using tetraethoxysilane (TEOS) derivatives [3].
The aim of this study was to synthesize a selective sorbent based on magnetic iron particles, which can be utilized in biomedical applications as selective sorbents for protein isolation.

Materials and Methods

All reagents of highest quality were purchased from Merck sp. z o.o. (Poland). Magnetic FeNPs were synthesized by co-precipitation process from aqueous solution. A solution of Fe^{2+} (0.027 M) and Fe^{3+} (0.023 M) was precipitated with concentrated ammonia. The aging of iron oxide sediments was carried out at room temperature for 60 minutes. The synthesized nanoparticles were thoroughly washed with a dilute ammonia solution (pH 10.5) and then with absolute ethyl alcohol containing concentrated ammonia using a magnet. The obtained FeNPs were redispersed in absolute ethyl alcohol. Then 1% v/v of each TEOS and concentrated ammonia as well as 2% of water were added to the suspension. The reaction was performed at room temperature overnight (≈18h). Next in the same manner the modification with 3-aminopropil-triethoxysilane was made. The obtained particles were washed with absolute alcohol and liofilized. The prepared particles were modified with succinic anhydride in dimethylformamide environment. The XRD technique was utilized for bare FeNPs characterization. Instead, both bare and functionalized particles were analyzied with SEM-EDX and ATR-FTIR techniques. Moreover, the sorption properties were studied by isotherm approach with the use of bovine lactoferrin standard and bicinchoninic acid method for protein quantification. Finally, the lactoferrin isolation was performed from skimmed bovine milk under the batch process in the phosphate buffer with NaCl gradient from 0,1 to 1M. The purity of the protein fractions were evaluated by SDS-PAGE technique.

Results and Discussion

Iron oxides of high magnetic properties has black colour. They are magnetite (Fe_3O_4) and γ-maghemite (γFe_2O_3). The mixture of Fe^{2+} and Fe^{3+} has orange colour, while addition of ammonia solution changed the colour of the solution into black indication the formation of magnetic nanoparticles. The XRD analysis revealed the formation of nanoparticles with crystallin structure. The comparative analysis with commercially available Fe_3O_4 nanoparticles (Sigma-Aldrich) shows the similar signals at 2θ nearly 30.5 (220), 35.9 (311), 43.5 (400), 53.8 (422), 57.4 (511), 63.5 (440), 74.5 (533). However, the differentiation between magnetite Fe_3O_4 and γ-maghemite γFe_2O_3 is quite difficult based on the XRD results [4]. We suggest that the mixture of both oxides are formed as the reaction was performed in the presence of oxygen. The SEM-EDX analysis with mapping revealed the formation of silica shell on the FeNPs core with evenly distributed functional groups. ATR-FTIR analysis revealed the appearance of new vibrational bands at nearly 2980 and 2880 cm^{-1} (CH_2), 1080 cm^{-1} (Si-O-Si), 960 cm^{-1} (Si-OH) on the spectrum of modified FeNPs. Study on the adsorption isotherm showed the high sorption capacity of the adsorbent. Finally, it was possible to obtain pure bovine lactoferrin by skim milk fractionation in the phosphate buffer with the NaCl gradient from 0.1 to 1.0M. The highest lactoferrin content was in the fraction with 1.0M of NaCl.

Conclusions

The selective adsorbent based on core-shell structure of magnetic Fe_xO_y nanoparticles was synthesized. The functionalization of iron oxide core was performed under Stöber process with the use of TEOS derivatives. The obtained sorbent revealed high sorption capacity against bovine lactoferrin ≈ 200 mg/g at pH =7.5. The study on the milk shows that it can be used for the pure bovine lactoferrin isolation.

Acknowledgements

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POROUS SCAFFOLD - TRANSFORMING WITH BLENDS

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Introduction

Bone is a dynamic tissue essential for locomotion, protection, mineral homeostasis. Bone tissue engineering, ideal for repairing defects, relies on seed cells, biocompatible scaffold materials, and biological factors. Bone scaffolds are 3D biomaterials used for defect reconstruction, and an ideal scaffold enhances cell adhesion, proliferation, differentiation, vascularization, host integration, and load bearing. It must be porous, bioactive, biodegradable, and mechanically strong. Polylactic acid (PLA) is a semicrystalline, biobased polyester with good biocompatibility and biodegradability. Incorporating ceramics can improve PLA's hydrophobicity, mechanical properties, and osteoinduction. β-Tricalcium phosphate (TCP) degrades at a rate similar to new bone formation. However, PLA's poor mechanical strength and low cell affinity limit its use in bone scaffolds. Polycaprolactone (PCL), known for its soft, elastic nature, promotes chondrogenic differentiation. Blending PCL with PLA reduces PLA's brittleness. Adding PEG as a compatibilizer enhances PLA and PCL blends. This study investigates the physicochemical and biological properties of PLA-based scaffolds with various compositions for bone tissue engineering.

Materials and Methods

Poly(lactic acid) (PLA, Ingeo Biopolymer 3052 D, Mn = 180 000) was obtained from Nature Works LLC, courtesy of Natur Tec Pvt Ltd, Chennai, Tamil Nadu. Poly (ε-caprolactone) (PCL, Mn = 80,000), poly(ethylene glycol) (PEG, Mn = 600 and Mn = 2000) were purchased from Sigma-Aldrich, Steinheim, Germany. Beta Tricalcium phosphate (β-TCP), analytical reagent grade, particle size < 500 µm) was provided from Sisco Research Laboratories Pvt. Ltd. Dichloromethane and chloroform were purchased from Merck KGaA, Darmstadt, Germany. The fabrication of the composite scaffolds was accomplished through a Gel foam casting technique utilizing a rapid heating method. Once the PLA/PCL/TCP blends reached homogeneity, the composite was gently poured over a preheated glass plate maintained at 70°C. The rapid evaporation of dichloromethane under these conditions led to the formation of random pores within the composite scaffold. Upon the complete evaporation of the solvent, a highly interconnected porous scaffold emerged, characterized by a homogeneous distribution of components. Notably, to assess the significance of PCL and PEG in the scaffold's structure, variations were introduced during fabrication while the TCP (w/w %) ratio was fixed for all the compositions.

Results and Discussion

PLA, a biodegradable polymer approved by the FDA, shows great potential for bone tissue engineering. Researchers have explored various forms, such as microspheres, membranes, and scaffolds. The gel casting method is commonly used to fabricate porous polymer scaffolds with pore sizes ranging from 100 to 1500 µm. Pore size significantly influences nutrient and cell movement, with larger pores promoting blood supply and bone in growth but reducing scaffold strength. Therefore, hierarchical pore architecture is crucial, as the macropore size of cancellous bone varies from 320 to 1670 µm. The presence of interconnected pores of size 100–1500 µm can be seen from the FIG. 1. The compression strength of 4.88 MPa was observed for the prepared PLA/PCL/TCP composite scaffold is within the range of compressive strengths reported for cancellous bone, which is the spongy bone found at the ends of long bones as shown in FIG. 2. This suggests that the material can withstand compressive loads similar to cancellous bone. These properties collectively determine the material's suitability for bone scaffolding applications.

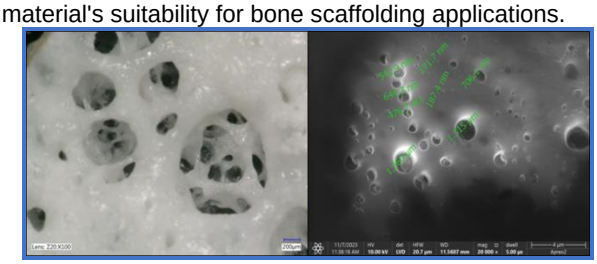

FIG. 1. Optical and SEM image of scaffold.

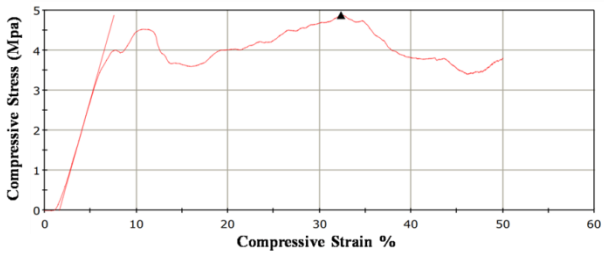

FIG. 2. Compressive stress-strain curve for the scaffold

Conclusions

This study aimed to fabricate polymer scaffolds using gel casting with rapid heating. PLA was blended with PEG and PCL to assess their influence on scaffold performance. TCP particles were embedded to enhance bioactivity. The scaffolds had pore sizes of 100–1500 µm. The addition of different polymers significantly impacted degradation rate, wettability, and thermal properties. Scaffolds with PCL showed reduced degradation due to its hydrophobicity, while PEG accelerated degradation due to its hydrophilicity. None of the scaffold extracts were cytotoxic to L929 fibroblasts and supported adhesion, proliferation of MG-63 cells. This fabrication method effectively produces highly porous, bioactive composite scaffolds with controlled degradation rates.

Acknowledgements

Indo-Poland joint grant project financed by, DST (DST Project No DST/INT/POL/P-41/2020), Govt. of India & by Polish National agency NAWA (Project No. PPN/BIN/2019/1/00083/U/000010).

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SCAFFOLDING OF A COMPOSITE FOR BONE TISSUE ENGINEERING

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Introduction

Biomaterials such as metals, ceramics, and polymers are extensively used in orthopedic applications to repair and replace damaged bone. However, traditional metallic implants, while mechanically strong, often suffer from bioincompatibility and inability to bond with bone tissue, leading to long-term complications such as implant loosening. To address these issues, bioactive glasses have been developed, which can bond with bone and degrade over time, allowing for new bone formation. This study focuses on the development of a metal oxide-doped fluorophosphate nanobioglass, designed to combine the beneficial properties of bioglass with enhanced mechanical strength and bioactivity.

The base glass composition includes phosphorus pentoxide (P_2O_5), calcium oxide (CaO), sodium oxide (Na_2O), and calcium fluoride (CaF_2). P_2O_5 acts as a network former, providing a bioresorbable matrix, while CaO contributes to the bioactivity and osteoconductivity. Na_2O helps lower the melting point of the glass, facilitating easier processing. The inclusion of CaF_2 introduces fluoride ions, known to enhance the rate of apatite formation and bone bonding. Metal oxide is incorporated as a dopant to improve the mechanical properties and chemical durability of the glass, making it more suitable for load-bearing applications in bone implants. This unique combination of materials aims to produce a nanobioglass that not only mimics the mechanical properties of natural bone but also supports bone regeneration through controlled degradation and bioactivity.

Materials and Methods

Metal oxide-doped fluorophosphate nanobioglasses were synthesized using the melt quenching method. The raw materials, including P_2O_5, CaO, Ag_2O, Na_2O, and CaF_2, were precisely measured, mixed, and melted at 1200°C. The molten glass was then annealed and cut into samples for analysis. Physical and thermal properties were assessed using techniques like ultrasonic study, Archimedes' principle for density measurement, and thermal analysis up to 1000°C. In vitro bioactivity was evaluated by immersing the samples in simulated body fluid (SBF) for 21 days, followed by characterization using FTIR, XRD, SEM, and EDS. Cytotoxicity was tested on AGS, MG-63, and SAOS-2 cell lines, while the biological efficiency was gauged by alkaline phosphatase activity and osteocalcin secretion assays.

Results and Discussion

The prepared nanobioglasses exhibited a Young's modulus of approximately 50-55 GPa, which is comparable to human cortical bone, reducing the risk of stress shielding. The addition of 5% TiO_2 improved the chemical stability and bioactivity, enhancing bone bonding. Fluoride incorporation at 2.5% further optimized the biodegradation rate, ensuring a balanced resorption with new bone formation. Hydroxyapatite formation was observed after 14 days of immersion in SBF, confirmed by FTIR peaks at 1030 cm^{-1} and XRD patterns matching standard hydroxyapatite. SEM analysis showed a uniform apatite layer formation, while EDS confirmed the presence of Ca and P in a ratio of 1.67, typical of hydroxyapatite. Cytotoxicity assays revealed over 90% cell viability, and osteogenic activity tests demonstrated a 1.5-fold increase in alkaline phosphatase activity and a 2-fold increase in osteocalcin secretion compared to controls.

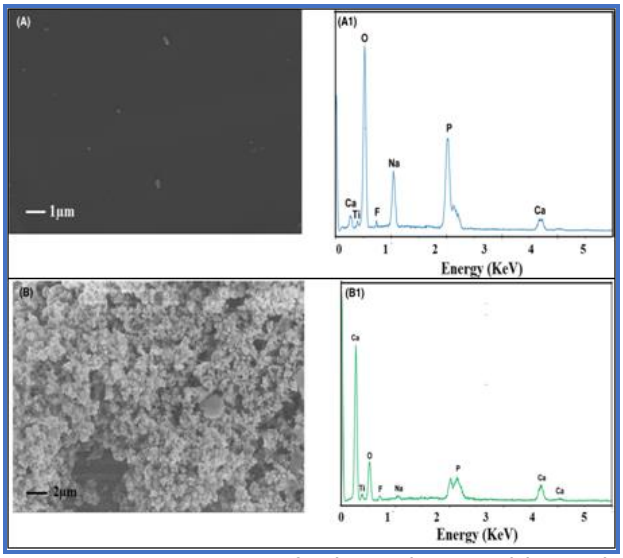

FIG. 1. SEM_EDS spectra for the pre immersed (A & A1) and post immersed (B & B1) sample.

Conclusions

Metal oxide-doped fluorophosphate nanobioglass, with a Young's modulus of 50-55 GPa and enhanced bioactivity, shows significant potential for use in bone implants. The material's optimized fluoride content ensures a biodegradation rate that aligns with bone regeneration, making it a promising candidate for orthopedic applications.

Acknowledgements

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Poli-IN - SYSTEM FOR CLOSING AN INEFFICIENT SAPHENOUS VEIN - FROM IDEA TO FIRST CLINICAL APPLICATION

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Introduction

Chronic venous insufficiency, the most common clinical form of which is varicose veins of the lower extremities - that is, congenital or acquired dysfunction of the venous vessels, caused by failure of the venous valves, is the most common vascular syndrome of the lower extremities [1]. The prevalence of chronic venous insufficiency in the adult population has been estimated at about 60%, with a far higher incidence in highly developed countries [2].
The following methods are used in the treatment of varicose veins: traditional, requiring hospitalization and rehabilitation (compression therapy, surgical removal of the diseased vein, closing the diseased vein using a thermal or non-thermal method) and innovative, based on the use of polymer glue to close damaged veins (VenaSeal, Medtronic; VariClose, Biolas; VenaBlock, Invamed).
A new medical device has been developed and tested in a preclinical study: the Poli-IN polymer adhesive, characterized by very good visibility in ultrasound monitoring of the procedure, achieved thanks to the contrast particles contained in the adhesive formulation.

Materials and Methods

An innovative formulation of a polymer adhesive based on cyanoacrylate (Poli-IN) was developed that provides specific quality criteria: polymerization time, good visibility during ultrasound examination, viscosity and density suitable for application through the originally designed catheter, as well as high polymerization efficiency. The n-butylcyanoacrylate/contrast agent mixture polymerization kinetics comparison to the reference product VenaSeal were studied by FTIR+ATR.
The physical and chemical properties of Poli-IN were evaluated:

- viscosity at 25ºC;
- polymerization time in the blood medium at 37ºC;
- adhesion to tissues in an in vitro test in a blood vessel taken from an animal under conditions of flowing animal blood (ASTM F 2255-05);
- polymerization structure under model conditions on blood flowing in a blood vessel model;
- strength of the occlusion breakage according to ASTM F 2458-05;
- heat polymerization by DCS;
- hydrolytic and enzymatic biodegradation in the SBF liquid according to ISO 10993-2, 13.

An appropriate panel of in vitro and in vivo tests was performed to confirm the biosafety of the Class III medical device in accordance with the guidelines of ISO 10993 and Pharmacopoeia, including evaluation of: cytotoxicity; intradermal reactivity; allergenic reaction (guinea pig maximization test); pyrogenicity; acute, subacute, subchronic, chronic toxicity (systemic and local); local reaction after implantation; genotoxicity (bacterial as well mouse cell mutagenicity); hemocompatibility (haemolysis, complement activation, APTT, PTT, platelet function, leukocyte count).
Material functionality and safety were evaluated in experimental animal studies, in particular: the precision of Poli-IN application procedure at the site of occlusion; the acute and chronic closure of the vessel; the acute and chronic toxicity; and the glue visualization quality in the place of location using ultrasound examination view.

Results and Discussion

It was confirmed that under laboratory conditions, the Poli-IN glue has a significantly shorter polymerization time than the VenaSeal reference material. On the basis of studies and laboratory tests additional supplements to the formulation of glue were developed:

- polymerization inhibitor to postpone the n-butylcyanoacrylate polymerization initiation, as well as
- homo-polymer synthesized from the base n-butylcyanoacrylate added to the glue mixture as a thickener that creates requested viscosity of the product and thus increases the surface tension of the glue droplet introduced into the venous vessel, thus delaying the rate of the glue polymerization course in the mass of the substance.

The average dynamic viscosity for the tested mixture is 48.2±0.6 mPa*s at 25°C. The polymerization time of the tested adhesive in the final formulation is 8 to 12 sec, respectively. In an in vitro evaluation of the adhesive's adhesion properties after polymerization, it was found that the Poli-IN adhesive with and without contrast agent additive had lower average rupture force values compared to the VenaSeal reference adhesive (12.7% less for the contrast-added material and 9.3% less for the contrast-free material). DSC analysis of heating the sample at a constant rate of 10°C/min to a temperature of 160°C showed the enthalpy value of the reaction, for this temperature, as 37.5 J/g. The biodegradation test confirmed that material does not degrade in period of 180 days.
The test material is not pyrogenic, does not exhibit genotoxicity, shows little irritation and mild sensitization. The detailed results of the haemolytic effects of the test article were analogous to or better than the results achieved in the same study with the reference VenaSeal. No systemic toxic effects of the material were found. Macroscopic evaluation at postmortem examination at 12 and 26 weeks after glue implantation showed no significant negative changes within the site of the material location and internal organs. An ultrasound inspection of the vessels 14 days after implantation confirmed the presence of polymerized glue in all the examined vessels. After 26 weeks of follow-up, the material was present in all blood vessels. No blood flow through the vessels was observed.

Conclusions

The results of the performed preclinical studies of the Poli-IN indicate the readiness to prepare clinical trials and confirm the safety of the substance and compliance with applicable legal and normative requirements. The research will continue within the framework of first-in-human use clinical trials, for which a positive opinion of the Bioethics Committee has been received.

Acknowledgements

Preclinical testing of the Poli-IN glue was conducted in cooperation with the Prof. Zbigniew Religa Foundation for the Development of Cardiac Surgery.

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FROM MULTIWELL PLATES TO BIONIC ORGANS. STEP FORWARD TO PERFUSABLE 3D TISSUE MODELS

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Abstract

3D models have become a major milestone in the development of modern science at all levels of its advancement. These models are used in basic research, where they enable more precise understanding of metabolic pathways and pathogenic mechanisms, as well as in developmental and industrial research, up to preclinical studies, where they are increasingly becoming alternative models to animal model research. Three-dimensional models produced using advanced tissue engineering techniques enable the development of structures that imitate the natural environment for cells. Equally importantly, the development of these structures introduces additional factors into the culture, both stimulating and stress factors. However, in the natural environment, cells are also exposed to them. The main factor stimulating the proper functioning of cells is bioink, which creates an environment for cell culture and growth enabling their proper functioning. On the other hand, stress factors include, for example, stress resulting from the applied pressure, cross-linking and flow.

Despite the great interest in flow models, 2D cultures are still an important point of scientific research. They are commonly used, among others, for screening studies. And they are certainly a valuable source of information for scientists in the initial stages of research. However, their major limitation is the fact that they do not reproduce the natural living conditions of cells. This is particularly important in the study of new medicinal substances or studies of cytotoxicity of biologically active molecules. Therefore, it is so important to develop more precise research methods and experimental models themselves so that the conducted research can best reproduce natural conditions and with the greatest possible accuracy allow the results of R&D and preclinical studies to be translated into their real application in practical medicine.

Certainly, 3D bioprinting is one of the fastest developing tissue engineering techniques for creating 3D models. However, it should not be noted that flow models currently take the same forms and different scales. Larger models are still a novelty. The most common are the so-called "organ-on-a-chip", which allow for conducting research on a micro scale. However, larger models (several centimeters) or entire organs (i.e. structures with an advanced vascular system and a much larger number of cells) can translate much more precisely into the effectiveness and reliability of the research conducted. Bioprinting of larger structures is revolutionizing modern science but also gives millions of people a chance. Because bioprinting goes a step further than just scientific, clinical or preclinical research. 3D bioprinting allows the production of organs and tissues that can be transplanted. Thus, it is becoming a technology with a much wider reach.

Initially, the use of 3D bioprinting to create models was associated with many limitations and problems at various levels of work. Starting from bioinks, programming g-code files, printing conditions (temperature, pressure) to the cross-linking process. Currently, these problems have been largely solved by many scientific teams around the world. And the only challenge apart from hardware limitations (bioprinters) is currently the cultivation of such models. Models that require a constant flow in controlled conditions. On the market, there are devices designed to test microcircuits or single systems of slightly larger sizes.

However, a future solution may be devices that are a kind of advanced incubators, which maintain sterility in their entire flow system and enable optimization of culture conditions in terms of temperature, pressure or flow rate as well as gas parameters. So far, the only such solution on our market is the Bioreactor for flow cultures enabling the cultivation of both large organs for transplantation and smaller models for research. Thanks to the applied automation and measurement regulation, the device enables standardized and repeatable culture processes, which in time may even be recognized as a certified research method. What is most important in this new solution, apart from standard culture parameters:

- pressure control - control of such a parameter is still particularly important in small flow systems. It ensures free flow of the medium while enabling quality control of the model and stability of its structure. Accurate and real measurement of this parameter protects the tested model from mechanical damage caused, for example, by too fast flow through the entire system.
- possibility of automatic exchange of the medium, which significantly reduces the possibility of infection of the tested models and organs.
- possibility of taking samples in a non-invasive way for the construct
- special chamber system, enabling bioprinting of models and their direct transfer to the culture device.

In summary, we are at the culmination of the development of tissue engineering, both in terms of biomaterials, technology (development of bioprinters and culturing devices) and cellular transformations, as evidenced by advanced work on stem cells and their application in clinical practice. The combination of knowledge of many scientists may soon contribute to a new face not only of the stage of preclinical research, which is now much talked about, but also of translational medicine. These activities conducted here and now are a milestone in translating scientific research from laboratory space into practical applications that will bring benefits to our health.

ADVANTAGES AND LIMITATIONS OF DRUG ENCAPSULATION IN POLYANHYDRIDE-BASED DRUG DELIVERY SYSTEMS

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Introduction

Polyanhydrides are an interesting group of polymers that gained interest in drug delivery due to rapid degradation of erosive mechanism. However, only one drug delivery system (DDS), Gliadel® - a carmustine-loaded wafer to treat malignant glioma, has been successfully translated into clinics [1]. Here, we present a study that focused on the limitations of polyanhydrides to encapsulate various drugs as one of the reasons for poor translation outcomes. Moreover, we present a few examples of drugs for which polyanhydrides exhibit a better performance than the other commonly used polymers thanks to drug-polymer conjugate formation.

Materials and Methods

Poly (sebacic anhydride) (PSA) and its 10% weight copolymers with poly(ethylene glycol) with M_w of 250 or 600 Da (PSAEG250 and PSAEG600, respectively) were synthesized by melt polycondensation and used as model polyanhydrides. The successful reaction was confirmed by ^{1}H NMR and FTIR. The thermal properties of the polymers were assessed by DSC and the hydrophilicity and surface free energy were evaluated by contact angle measurements with water and diiodomethane. Drug-loaded microparticles (MPs) were fabricated from the three polymers and four model drugs: gentamycin, free (GN) or hydrophobized by hydrophobic ion-pairing (hGN) [2], azithromycin (AZ), curcumin (CU), and linoleic acid (LA). The MPs were manufactured by single or double emulsification (O/W or W_1/O/W_2, respectively), where W_1 was an aqueous solution of water-soluble drug, O was a solution of polymer with dispersed drug (free for water-insoluble substances or as a water solution for water-soluble substances), and W_2 was a poly(vinyl alcohol) (PVA) solution in MilliQ water. The successful MPs fabrication was confirmed by SEM. The encapsulation efficiencies (EE) and drug loadings (DL) were assessed by direct fluorometric assay (CU) [3], OPA-assay (GN, hGN) [2], or mass spectrometry (MS) (AZ) [4]. For analysis of the conjugates, the MPs were dissolved in chloroform, diluted 100 times, and injected directly into the mass spectrometer to detect the ions of conjoined drugs and monomers. The analysed ions were separated and fragmented to ensure their conjugate origin. To check the potential disturbance of the process on the bioactive properties, AZ-loaded and hGH-loaded MPs were tested with *Staphylococcus aureus* and the release of the pure drug in biological environment was tested by incubation with human microsomes.

Results and Discussion

The synthesized polymers were crystalline solid materials with cream yellow colour. The glass transition was observed in the range between -55 and -30°C and the melting temperatures were in the range of 60-85°C. The materials exhibited high hydrophobicity that, however, was lower for PSA copolymers with PEG, than for PSA.
The fabricated MPs were spherical (PSA, PSAEG250) or elongated rode-like (PSAEG600) with most diameters in the range of 0.5-3 µm. All the samples were white, apart from CU-loaded MPs which were yellow due to the presence of the drug. Encapsulation evaluation showed that the encapsulation of free GN was below detection limit while for hGN it was satisfactory. Higher EE values were obtained for copolymers rather than for pure PSA. Also for LA, only trace amounts of the substance were entrapped within the MPs. EE of AZ was nearly 100% while for CU-loaded it was up to 55%. Again, PSAEG250 and PSAEG600 performed better than PSA. MS analysis showed conjoined drug molecules with up to several monomers at a time with AZ and CU. For AZ, the conjugation with polymeric matrix occurred with the esterification reaction yield of 100%, explaining such a high EE. The encapsulated CU was partially reacted with the polymer as well, however, a considerable amount of the drug was in its free form, as confirmed by MS analysis. Isolation and fragmentation of the ions confirmed their conjugate origin. In turn, incubation with human microsomes showed gradual detachment of the conjugates in the biological environment. hGN-loaded and AZ-loaded MPs effectively inhibited the growth of *S. aureaus*, as well.
The EE showed that the main problem of encapsulating drugs in polyanhydride MPs is their high crystallinity and chemical incompatibility when it comes to the hydrophilic drugs. However, the first of the mentioned limitations has a high impact, as hydrophobization did not improve the EE spectacularly, as it was previously noticed for less crystalline polymers, e.g. PLGA [2]. Also, naturally hydrophobic LA exhibited very poor EE.
Hydrophobic drugs with hydroxyl groups in their structure may be efficiently encapsulated due to the esterification reaction. The reaction is extremely effective for polymers containing alcohol groups and less effective for those containing phenol groups as they are naturally less reactive in esterification formation. Moreover, the drugs do not lose their antibacterial properties during the manufacturing process and can be released as pure substances in the human body.

Conclusions

Drug-polymer conjugates between polyanhydrides and hydrophobic alcohols are the most promising combinations for designing DDSs with the use of these polymers.

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BIONEUTRAL, HEMOCOMPATIBLE OLIGOPROLINE COATINGS EVALUATION TOWARDS APPLICATION IN POLISH HEART SUPPORT SYSTEMS FOR CHILDREN

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Introduction

The number of patients with heart failure has been steadily rising over recent decades. In the United States, over 5.7 million people are now affected, with more than half dying within five years of diagnosis. In Poland, approximately 1.24 million people, or 3.2% of the population, suffer from heart failure. Alarmingly, the incidence of heart failure in children, as well as the number of children requiring heart transplants, continues to grow. In the United States alone, it is estimated that each year, 12,000 to 35,000 patients under the age of 19 are diagnosed with heart failure. The lengthy wait for an organ and the differences in anatomical and physiological characteristics based on age result in a high mortality rate within this group. Unfortunately, the COVID pandemic has also contributed to the expansion of the number of patients suffering from this disease syndrome. The impact of COVID-19 on the heart is especially dangerous for people with pre-existing heart disease, as it can lead to acute or chronic heart failure, which can be life-threatening. Pulse pumps are also the only alternative to heart transplantation in children. Currently, the third-generation fully implantable centrifugal pumps, most commonly used in adults, have very little use in children due to the limited space in the chest. To address this issue, the Foundation of Cardiac Surgery Development (FRK) is conducting research and development aimed at creating a Polish family of extracorporeal paediatric pumps for cardiac support, with the goal of preparing them for clinical use. Despite the advanced pharmacotherapy and innovative biomaterial procedures, due to the complexity of coagulation processes, the main medical problem of pulsatile VAD is susceptibility to forming blood clots due to non-physiological dynamics of blood flow in artificial chambers. The overall incidence of thrombosis in VAD flow pumps is reported to be even up to 30%. What is a very important risk of pump thrombosis is the highest within the first few days to months after VAD implantation. The first 72 hours after implantation play a key role, because due to the risk of postoperative hemorrhages, the prevention of anticoagulant therapy is limited during this period. One of the solutions may therefore be the optimization of materials in contact with blood, considering new surface engineering techniques, and ensuring a high biosafety factor, including hemocompatibility.

Materials and Methods

International cooperation allowed to develop coatings based on oligoproline directly on biocompatible PU and an intermediate silver buffer layer, which are both antibacterial and minimally activating blood. As part of the preliminary work, the assessment of physico-chemical properties obtained with selected material engineering methods and hemo-compatibility analysis were performed. Assessment of the surface topography of the coatings was carried out, among others using microscopic diagnostic methods, i.e. a digital microscope Keyence VHX-5000 and a scanning electron microscope ThermoScientific Scios 2. In addition, the analysis was extended to the use of the MAHR XR1 contact profile and evaluation of the wettability of modified surfaces using an optical goniometer. In-situ mechanical testing was conducted in a scanning electron microscope (SEM) chamber using a device manufactured by the Swiss company ALEMNIS. This setup enabled the performance of a scratch test while simultaneously observing the phenomena occurring on the material's surface using an electron beam. A Rockwell C indenter with a diameter of 100 µm was employed for the test. Biodegradation studies were performed under three conditions: atmosphere, PBS solution and desiccator. Biological research included, among others, the assessment of cytotoxicity and hemocompatibility in static and dynamic conditions, based on, among others, flow cytometry and CLSM using ZEISS LSM Exciter 5.

Results and Discussion

The use of oligoproline as a surface modification for polyurethane in cardiac support devices offers a novel solution to reducing blood clot formation, a major challenge in such applications. Oligoprolines (Pro6 and Pro9), modeled after collagen's polyproline type II (PPII) helix, have shown promising results. Pro9, with a more stable structure, may be particularly effective in clinical conditions. Experimental data showed that Pro6 and Pro9 have a contact angle of ~25 degrees with water, indicating strong hydrophilicity similar to that of biocompatible polyethylene glycol (PEG). This hydrophilic nature helps reduce protein adsorption and platelet adhesion, key factors in preventing thrombus formation. Cytotoxicity tests confirmed the safety of oligoproline-modified surfaces, with cell viability exceeding 99.5% after 24 hours, indicating strong biocompatibility. Additionally, partial thromboplastin time (PTT) tests demonstrated extended coagulation times compared to reference materials, suggesting these surfaces significantly lower the risk of clot formation, a crucial factor for the safety and longevity of cardiac devices.

Conclusions

Preliminary studies have confirmed the possibility of developing coatings based on oligoproline, limiting susceptibility to adsorption of protein and serum components, which can be of key importance in the first days when supporting circulation in patients. The incorporation of oligoproline into polyurethane surfaces for cardiac devices not only enhances hemocompatibility by reducing the risk of blood clot formation but also maintains high biocompatibility, making it a promising solution in the field of cardiovascular medical devices.

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ANTIBACTERIAL AND OSTEOGENIC CALCIUM CARBONATE-BASED MICROPARTICLES FOR BONE TISSUE REGENERATION

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Introduction

Calcium carbonate ($CaCO_3$) microparticles are extensively researched as drug delivery carriers due to their excellent biocompatibility, degradability, and porous microstructure allowing for successful loading with various drugs or biologically active molecules [1]. One of the area in which these microparticles can be used is in the treatment of bacterial infection and supporting bone tissue regeneration [2]. Considering growing bacteria resistance to conventional antibiotics, other promising antibacterial agents, such as antimicrobial peptides (i.e. bacitracin), gained a lot of interest recently.

The aim of this study was to synthetize highly porous $CaCO_3$ microparticles and evaluate their suitability for bacitracin (BCT) adsorption and utility in terms of dual action drug delivery systems: antibacterial and osteogenic. In the course of study, the microparticles' structure was modified with the addition of zinc ions (Zn^{2+}) to improve BCT binding capacity.

Materials and Methods

$CaCO_3$ microparticles were obtained using precipitation method from aqueous soluble Na_2CO_3 and $CaCl_2$ salts. Three different mixing speeds were tested to obtain microparticles differing in size. $CaCO_3$ microparticles were evaluated in terms of their morphology (scanning electron microscopy – SEM), particle size, and surface area (Brunauer–Emmett–Teller method – BET). BCT was adsorbed on the surface of microparticles from aqueous solution. BCT adsorption efficacy was measured by spectrofluorimetric method using *o*-phtaladehyde (OPA assay).

The second part of the study focused on the microparticles with Ca^{2+} partially substituted with Zn^{2+} (up to 20% of initial $CaCl_2$ solution was replaced with equimolar $ZnCl_2$ solution). The obtained $(Ca/Zn)CO_3$ microparticles were characterized in detail using SEM coupled with EDX analyser, BET, particle size measurements and X-ray diffractometry (XRD). BCT was adsorbed on the microparticles in the same manner as in pure $CaCO_3$ microparticles. The modified microparticles were evaluated in terms of microstructure, BCT adsorption efficacy, BCT release and biological activity (cytotoxicity in contract with L292 mouse fibroblasts, antibacterial activity towards *Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pyogenes,* and osteogenic differentiation of human mesenchymal stem cells – hMSC).

Results and Discussion

All $CaCO_3$ microparticles were spherical in shape and highly porous, resembling vaterite morphology, however their size increased with the decrease in mixing speed during precipitation. BET surface area significantly increased with the decrease in particle size, which resulted in more efficient BCT adsorption in smaller microparticles. The maximum BCT content in microparticles was around 1%, which was below expectations and could not be regarded as useful in terms of future use.

The addition on Zn^{2+} significantly influenced the morphology and surface area of the microparticles, as even though the semi-quantitative EDX analyses showed that maximum content of Zn was below 1% at., the microparticles containing Zn were larger, less porous and with calcite crystallographic structure. Nonetheless, the adsorption capacity of BCT improved remarkably, reaching around 40% of BCT loading in the case of the microparticles with the highest Zn content. BCT was almost completely released from the microparticles within the first 24 h of incubation in phosphate-buffered saline, and it was still active as evidenced during microbiological study. The microparticles containing the highest amount of Zn were cytotoxic towards both fibroblasts and hMSC, whereas $(Ca/Zn)CO_3$ microparticles containing fewer amounts of Zn were cytocompatible. Preliminary studies showed that the microparticles stimulated osteogenic differentiation of hMSC, however this effect was more likely attributed to the release of Zn^{2+} than to BCT.

Further studies will be performed to explain the nature of the binding between $(Ca/Zn)CO_3$ microparticles and BCT, as well as to fully characterize their influence on hMSC to fully evaluate their potential bone tissue regeneration.

Conclusions

Uniform and highly porous $CaCO_3$ microparticles were fabricated using simple precipitation method allowing for control over particle size by varying mixing speed during the reaction. Despite a large surface area and microstructure suitable for adsorption, BCT binding capacity of pure $CaCO_3$ microparticles was below expectations.

The developed $(Ca/Zn)CO_3$ microparticles had been proved to effectively bind BCT and to release it in a rapid manner allowing effective prevention of bacterial growth. Zn-containing microparticles supported osteogenic differentiation of hMSC, which make them promising dual action drug delivery carriers to be used in the field of bone tissue regeneration.

Acknowledgements

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HOLLOW ALGINATE CAPSULES MODIFIED WITH SILVER AND COPPER NANOPARTICLES FOR THERANOSTIC APPLICATIONS

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Introduction

In theranostics, therapy and diagnostics—including drug delivery and monitoring of treatment response—can be integrated to provide targeted, personalized treatment. This approach has become particularly promising in oncology, where identifying specific molecular targets and delivering targeted therapies precisely can significantly improve cancer treatment outcomes while minimizing side effects. Various materials tested as potential theranostic agents include superparamagnetic iron oxide nanoparticles, gold, silver, ceramic, and polymeric nanoparticles. Among these, polymers, including hydrogels such as sodium alginate, offer unique versatility. Recently, significant attention has also been focused on organic-inorganic hybrid systems, where certain metal nanoparticles can enhance the properties of a hydrogel carrier. Therefore, the hybrid system should not only be capable of encapsulating and releasing drugs but also possess suitable detection capabilities for biological markers, potentially through surface-enhanced Raman spectroscopy (SERS) [1].

In this research, advanced particles intended for theranostic applications, based on hollow hydrogel capsules constructed using calcium carbonate templates and modified with silver and copper nanoparticles, were designed, synthesized, and characterized.

Materials and Methods

To produce $CaCO_3$ template particles, appropriate volumes of 0.33 M solutions of sodium carbonate (Na_2CO_3 anhydrous pure p.a., CHEMPUR) and calcium chloride ($CaCl_2$ anhydrous pure p.a., POCH) were placed in a 10 ml glass beaker and either stirred vigorously with a magnetic stirrer at 500 rpm (for bigger particles) or sonicated (Sonics, VibraCell) at a frequency of 20,000 kHz (for smaller ones) for 1 minute. The precipitated $CaCO_3$ particles were centrifuged (2000 rpm, 2 minutes), washed three times with 70% ethanol (v/v), and then dried in a laboratory dryer at 60°C for 30 minutes.

Hollow alginate capsules were prepared in two sizes based on the initial size of the obtained $CaCO_3$ template particles. 1 ml of a 5 % sodium alginate solution was added to each tube containing the $CaCO_3$ powder. After 10 minutes of shaking, the coated $CaCO_3$ particles were rinsed three times with deionised water and centrifuged at 2000 rpm for 2 minutes. Following this, 0.5 ml of a 0.75 M $AgNO_3$ solution or a 0.75 M $CuSO_4$ solution was added. The mixture was again shaken for 10 minutes and then washed three times with deionised water. In the final step, 0.1 M ascorbic acid solution was introduced to the system to remove the $CaCO_3$ template, resulting in the formation of silver/copper alginate hydrogel capsules. Finally, the formed capsules were centrifuged and washed three times with deionised water at 2000 rpm for 2 minutes. The silver/copper alginate hydrogel capsules were stored in deionised water at room temperature.

Morphology, microstructure, chemical composition, size distribution, structure through FTIR-KBr and XRD were analyzed to characterize obtained particles.

Results and Discussion

$CaCO_3$ templates were produced in two sizes – bigger particles with a mean diameter of 3.34 ± 0.62 µm and smaller particles with a mean diameter of 1.22 ± 0.25 µm (FIG. 1). The XRD diffraction patterns indicated that calcite was more pronounced in the *b*_$CaCO_3$ sample, while vaterite dominated in *s*_$CaCO_3$. These findings corroborate the SEM and FTIR results, highlighting that despite different synthesis techniques, both calcium carbonate samples contained a coexistence of various crystallographic forms. Overall, smaller calcium carbonate particles were recognised as more promising due to their better size distribution, uniform morphology, and round shape.

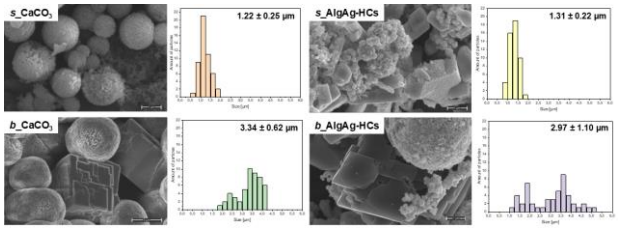

FIG. 1. Representative SEM images and histograms of particles diameters for smaller and bigger $CaCO_3$ templates and silver alginate (AlgAg) hollow capsules (HCs)

The introduction of silver and copper ions into the alginate capsules affected the visual characteristics of the materials, changing their colour to black and yellowish, respectively. The smaller s_AlgAg hollow capsules (1.31 ± 0.22 µm) exhibited a more uniform size distribution compared to the larger b_AlgAg-HCs (2.97 ± 1.10 µm), despite their greater tendency to aggregate (FIG. 1). The morphology of AlgAg and AlgCu hollow capsules was different from the spherical s_$CaCO_3$ particles – uniform nanoparticles of silver or copper were present on the surface of HCs.

SEM/EDS analysis also confirmed the presence of residual $CaCO_3$ in both *b*_AlgAg-HCs and *s*_AlgAg-HCs, along with sodium from the sodium alginate used in the coating process. FTIR-KBr results confirmed the successful modification of all particles. The XRD analysis allowed to define the phase composition of the synthesised particles.

Conclusions

The results indicated that AlgAg-HCs based on smaller $CaCO_3$ particles were the most promising candidates for further development. These capsules had the least residual $CaCO_3$, the best size distribution, and a desirable morphology. The same hybrid system is currently being developed as a coating for magnetic-core nanoparticles.

Acknowledgements

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FEM ANALYSIS FOR BONE-LCP JOINT

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Introduction

Nowadays, more and more importance is attached to technological excellence innovation in the field of medicine. The life span of a person has increased significantly, leaving behind the evolution of the skeletal system, resulting in the appearance of diseases such as osteoporosis. It is a disease of affluence, during which, as a result of aging weakening and atrophy of bone tissue. In addition, the increasing number of accidents, especially traffic accidents and accidents related to with them injuries of the musculoskeletal system have significantly increased the demand for all types of implants and prostheses. Bone anastomoses are a serious challenge for doctors, especially when the bone is also osteoporotic. Previous research on innovative tiles LCP (Locking Compression Plate), which are equipped with a special locking mechanism, show their superiority over traditional methods. They are characterized by very good bone stabilization, minimalization of movement around the fracture site, faster bone fusion and, consequently, a shorter recovery period for the patient and a longer a chance to return to full mobility.
The aim of modern orthopaedics is to achieve full functionality and a quick recovery period. The main focus of the paper is the FEA (Finite Element Analysis) for bone anastomosis using an LCP.

Materials and Methods

The strength analysis of the bone-plate model LCP was performed in the Inventor Environment Professional 2023, by Autodex. The Element Method was used in the study Finite Element Method (FEM).
The material underlying the bone model was medical record, containing X-rays of 3 patients, which was used for further research.

Results and Discussion

The subject of considerations in the paper was the selection of parameters and materials for the LCP board fusing the bone for this purpose, the case of bone fixation with an LCP plate for bone fracture was modelled sagittal. The influence of the selection of parameters and the structural material of the board condition of tensions and displacements in the bone-plate system, assuming that the entire body weight. It is transmitted, while standing by one of the lower limbs. For each of the analysed titanium alloys, the results of analyses with a similar course were obtained. Maximum and minimum values occur in the same places, assuming similar values.
The maximum values of reduced stresses in bone-plate systems have always been present at the point, where the top screw is connected to the board. The results of the study suggest defectiveness of the designed screw connections due to the occurrence of exceeding the permissible stress values for each elements of the plate-bone assembly.
In the model, the tensile strength limit values were not exceeded specified in Bedzinski [1]. Division the model with different strength properties allowed for an analogous simulation to the anatomical structure of bones.

Conclusions

The tests performed showed, that the lowest stresses reduced occurred, when titanium alloy plate Ti-13Nb-13Zr was used. Simultaneously it was in the case of this plate, that the largest total displacements occurred, but compared to the results for the study of the LCP bone and plate system made of Ti-6Al-7Nb alloy, which obtained the lowest values of total displacements, the difference is 0.0191 mm for the chip model and 0.0024 mm inserts. However, in the study on the use of Ti-6Al-7Nb material, the stress achieved the highest values among the three analyzed materials. Values intermediate in both of these criteria was obtained by the LCP bone-plate system made of titanium alloy Ti-6Al-4V, but its presence in the study was only a control, as due to the strong carcinogenicity, even with short-term contact with the body, should not be
be used on implants.

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FAST SCREENING BREATH ANALYSIS FOR DIAGNOSTICS OF PULMONARY ARTERIAL HYPERTENSION

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Introduction

Pulmonary arterial hypertension (PAH) is a rare disease with a serious prognosis. Unfortunately, it is often diagnosed in the late phase as its symptoms are non-specific and there is a lack of screening tests. Therefore, there is a strong need to identify of its biomarkers. In our research, we have examined the breath phase of patients with PAH that was collected using a special patented holder containing the highly porous organic and highly surface-developed pure carbon septic material as well as the serum. The collected air consisting biomarkers was surveyed with headspace analysis by the use of gas chromatography-mass spectrometry (GC/MS).
Due to preliminary results, the suggested method seems to be specific and sensitive in the range of selected biofingerprints. During the method development, we examined several patients with diagnosed PAH as well as healthy patients as a reference group.

Material and Methods

The breath phase of all the patients was collected on the highly porous septic material by the use of a special patented holder. The collected air was then examined with (GC/MS). A group of 10 patients (2 men, 8 women, mean age 60.4 ± 10.9 years, BMI 27.6 ± 6.0 kg/m^2) with diagnosed PAH as well as the group of 10 healthy persons (6 men, 4 women, mean age 35 ± 11 years, BMI 25.6 ± 6.0 kg/m^2) were enrolled into the study. For 4 PAH patients, the root cause of the disease was congenital heart disease (3 cases with ASD II, 1 case with atrioventricular septal defect) with clinical phenotype of Eisenmenger syndrome, for another 1 systemic sclerosis, and in 3 cases the cause was idiopathic. The examined patients also suffered from the following chronic diseases: 4 from arterial hypertension and 2 from diabetes. None of the patients were currently a smoker, but 3 of them had an anamnesis of smoking in the past (>10 years ago).
The obtained spectral and chromatographic results clearly present the qualitative and quantitative QA/QC sensitivity to the metabolite changes in the patient's breath. The identification of changes in the ratio of the whole spectra of biomarkers can allow us to obtain a multi-dimensional pathway for PAH diagnostics FIG. 1.

Conclusions

The suggested method, due to preliminary results, seems to be specific and sensitive in the range of selected bio-fingerprints. The molecular level breath analysis can be used as a screening test as well as a complementary diagnostic method to the standard procedure.

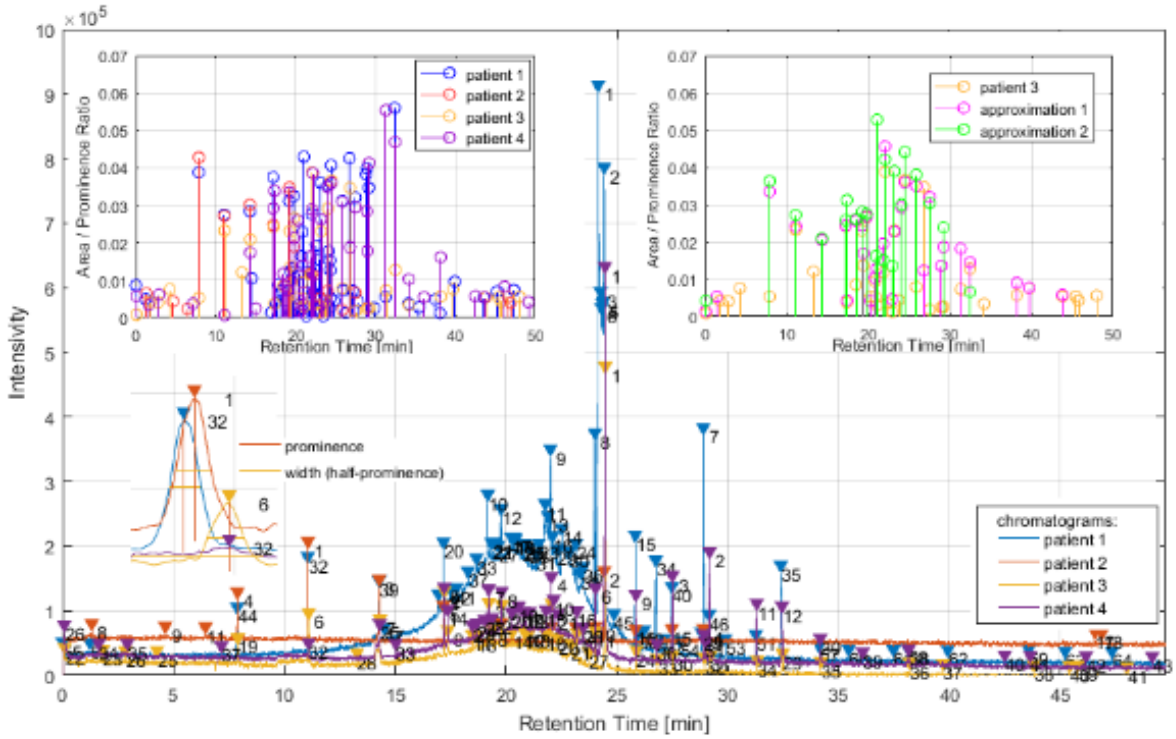

FIG. 1. The chromatograms of four patients suffering from pulmonary hypertension with distinguished significant peaks (numerate in the descent way according to their prominence). On the left side, there are the graphical presentations of the area under the peak to its prominence ratio, for all four patients. On the right side, there is also the same graphical presentation (area/prominence for peaks) of the arbitrarily chosen patients, but with reference to the original data and for two data approximations obtained by the DWT, which are important in the case of weak separated peaks.

INNOVATIVE APPLICATION OF POROUS CARBON MATERIAL FOR DETECTION OF LARYNGEAL CANCER THROUGH EXHALED BREATH ANALYSIS

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Introduction

Early detection of cancer is critical for improving prognosis and survival rates. Traditional screening methods for respiratory tract cancers, such as imaging and invasive biopsies, often present limitations in terms of practicality, reliability and patient compliance. Detecting volatile organic compounds (VOCs) and other biomarkers in exhaled breath represents a promising, non-invasive alternative with potential for rapid and accurate diagnosis. Metabolomic analysis of exhaled breath has already demonstrated success in identifying diseases such as asthma [1] and pulmonary arterial hypertension (PAH) [2], highlighting its potential applicability to cancer detection.
This study aims to develop and assess an innovative screening methodology for the detection of laryngeal cancer, utilizing highly porous carbon material for the collection of volatile organic compounds.

Materials and Methods

The study involved a group of 20 participants, consisting of 10 healthy individuals and 10 diagnosed with laryngeal cancer. Breath samples were collected using a highly porous carbon material [3] developed in 2015 by a research team led by dr hab. Andrzej Swinarew, prof. UŚ. This material, based on cyclic potassium glycidoxide hexamer, features a unique structure that significantly enhances the capture of VOCs through both absorption and adsorption processes, requiring fewer exhalations than conventional gas sampling bags.
Participants followed a standardized sample collection protocol: rinsing their mouths with water to eliminate food residue impacts, then exhaling into sterile tubes containing the carbon material for at least 3 seconds per exhalation, repeated ten times. The collected samples underwent thermodesorption at 37°C for 30 minutes using a Shimadzu AOC-5000 Plus PAL autosampler, followed by gas chromatography-mass spectrometry (GC-MS) analysis with a Shimadzu GCMS-QP2010 Plus equipped with a ZB 5MSI column. The GC-MS protocol initiated at 36°C, held for 1 minute, and then increased at a rate of 8°C per minute up to 250°C, where it was maintained for 25 minutes.

Results and Discussion

Chromatographic analysis revealed distinct differences in VOC profiles between cancer patients and healthy individuals, with significantly higher peak intensities observed in the group with laryngeal cancer (FIG. 1). These preliminary findings suggest that the novel carbon material-based method, encompassing both sample collection and subsequent analysis, demonstrates promise in potentially distinguishing between cancerous and non-cancerous respiratory conditions. The method's apparent efficiency in capturing a broad range of biomarkers underscores its potential utility in clinical screening settings.

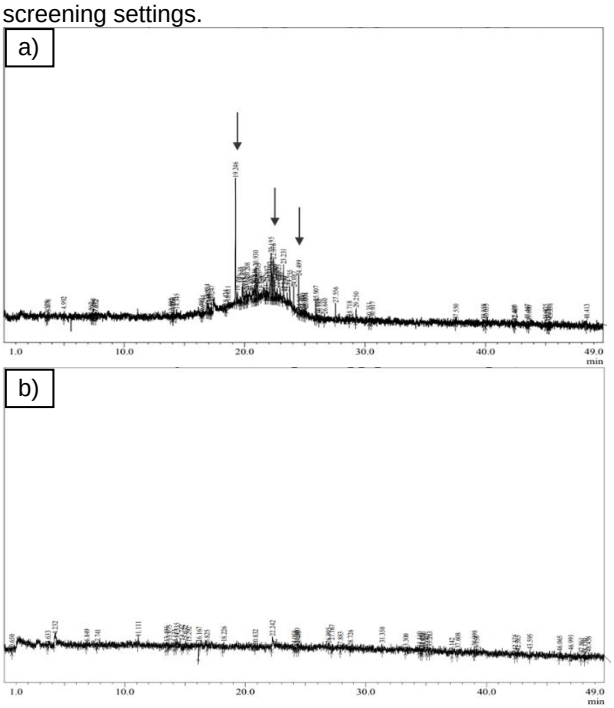

FIG. 1. a) Chromatogram of a patient with laryngeal cancer; b) chromatogram of a healthy individual

The observed differences in VOC profiles also raise the possibility of utilizing pattern recognition algorithms or machine learning techniques to enhance the diagnostic accuracy of the screening method. By analyzing the collective VOC signature, rather than individual compounds, it may be possible to improve the sensitivity (up to 0,1 ppb) and specificity of laryngeal cancer detection, potentially leading to earlier diagnosis and improved patient outcomes.
However, it is essential to acknowledge the limitations of this preliminary study, including the small sample size and the need for validation in larger study groups. Additionally, further research is warranted to elucidate the specific VOCs responsible for the observed differences and to optimize the methodology for routine clinical use.

Conclusions

The developed carbon material-based method shows promise for early laryngeal cancer detection based on the observed differences in volatile and semi-volatile organic compound profiles. However, addressing the study's limitations, such as the small sample size, and validating the methodology in larger study groups are necessary for further confirmation. Future research efforts should focus on refining the methodology and identifying specific biomarkers for clinical applicability. This study marks an initial step towards non-invasive cancer detection, with potential implications for improving patient outcomes through early intervention.

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SURFACE MODIFICATION OF PURE MAGNESIUM TO INHIBIT EARLY-STAGE RAPID CORROSION AND TO IMPROVE OSTEOBLAST COMPATIBILITY

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Introduction

Titanium and its alloys are clinically used as substrates for bone fixation devices due to their high corrosion resistance, and osseointegration property [1,2]. However, titanium requires surgical removal with high invasiveness and an extended period for full recovery [3]. Recently, magnesium (Mg), a biodegradable metal, has been garnering attention as a substrate for bone fixation devices. Because pure Mg degrades very quickly with generating hydrogen gas, Mg alloys such as WE43 with corrosion resistance are used as a substrate for medical devices such as cardiovascular stents and bone fixing screws [4]. In the case of Mg alloys, the toxicity of additional metals is a significant concern [5]. In this study, we investigated the surface modification of pure Mg with bone morphogenetic protein through a polydopamine layer to inhibit early corrosion and improve the compatibility of osteoblasts.

Materials and Methods

Mirror-polished pure Mg plates (99.95%) were immersed in 1 M NaOH solution for 24 h at 50°C and then washed with pure water [6]. Subsequently, the Mg plates were immersed in 20 mM dopamine/0.1 M NaOH solution for 24 h at 50°C, washed with pure water, and dried [7]. The surface morphology of pure Mg plates with or without modifications was observed using a 3D laser microscope. The element composition of the pure Mg surfaces was analyzed by X-ray photoelectron spectroscopy (XPS). The corrosion degradation behavior of the pure Mg plates in tissue culture medium (αMEM) containing 5% FBS was analyzed electrochemically by tripolar polarization measurements using stainless steel as the counter electrode. In addition, bone morphogenetic protein (BMP-2) was covalently immobilized onto the surface of pure Mg plates through the polydopamine treatment, and the adhesive behavior of mouse osteoblast-like cells was evaluated *in vitro*. Furthermore, porous scaffolds consisting of pure Mg wire with polydopamine treatment were fabricated and transplanted into the defects created in rat skull.

Results and Discussion

The crystal grain boundaries of pure Mg disappeared in 3D laser microscopic observations after polydopamine treatment. In the XPS analyses, nitrogen atoms derived from polydopamine were strongly detected, suggesting successful coating of polydopamine onto the surface of pure Mg using 1M NaOH solution (FIG. 1A). The results of electrochemical corrosion decomposition test in αMEM showed that not only the initial corrosion but also the late-stage corrosion of the pure Mg plates was strongly inhibited by the polydopamine coating (FIG. 1B). We anticipate that the gaps in the polydopamine layer were filled during the initial corrosion, leading to the formation of a stable passive layer that suppresses the late-stage corrosion of pure Mg plates.

Non-treated pure Mg plates exhibited significant corrosion and fragmentation after 48 hours (FIG. 2). On the other hand, polydopamine-coated pure Mg plates managed to maintain their morphology even after 48 hours. Furthermore, the adhesion of mouse osteoblast-like cells was slightly enhanced on pure Mg surfaces with the immobilization of BMP-2 through the polydopamine layer. In the *in vivo* experiment, slight bone regeneration was observed around the pure Mg scaffolds with polydopamine coating after 3 months of transplantation. Our hypothesis is that the polydopamine coating suppressed the immediate generation of hydrogen gas due to the drastic corrosion of the pure Mg scaffolds after transplantation.

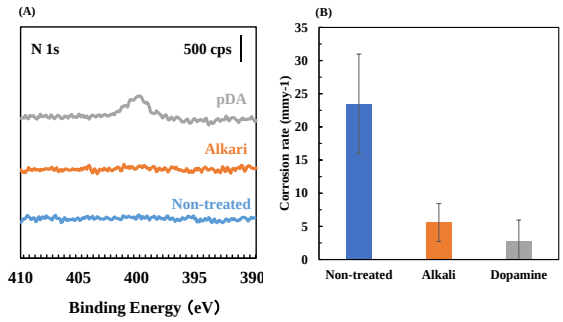

FIG. 1. (A) XPS analysis of pure Mg plates with polydopamine treatment. (B) Corrosion rate of Mg plates in tissue culture medium determined from polarization curves.

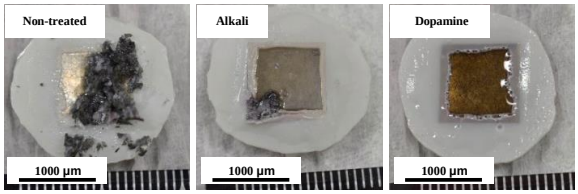

FIG. 2. Appearance of the corrosion behavior of pure Mg plates in tissue culture medium after 48 hours.

Conclusions

In this study, we successfully demonstrated that polydopamine coating effectively inhibited the corrosion of pure Mg. However, the additional immobilization of BMP-2 was not effective in improving the compatibility of osteoblast-like cells. In the future, we will investigate the enhancement of bone compatibility of pure Mg through the co-immobilization cell adhesive molecules with BMP-2 through polydopamine coating.

Acknowledgements

We would like to thank Prof. Yoshiya Hashimoto, Dr. Kenichiro Yasui and Takeryo Adachi in Osaka Dental University (Japan) for technical support *in vivo* experiments.

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CORROSION BEHAVIOR OF Ca-P COATING PREPARED ON MAGNESIUM ALLOY BY PLASMA ELECTROLYTIC OXIDATION

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Introduction

Every year, the number of bone fractures resulting from accident or illness is increasing. Analyzing global statistics, there are 178 million new fractures in 2019 [1]. In the case of more complicated fractures, surgical intervention may be necessary and the placement of internal stabilization using various types of bone plates, screws or intramedullary nails. Once the fracture has healed, these implants must be extracted, which involves a second operation that raises the risk of infection and puts a strain on the patient's body. Hence, research work is being undertaken to develop a biocompatible material that will undergo controlled degradation and resorption after implantation.

Magnesium is easily corroded in physiological environments due to its chemical properties and can degrade. Magnesium dissolution in an aqueous environment usually proceeds through an electrochemical reaction with water, resulting in the formation of magnesium hydroxide $Mg(OH)_2$ and hydrogen gas H_2. This phenomenon is described by the following equations:

Anodic reaction: $Mg \rightarrow Mg^{2+} + 2e^-$ (1)

Cathodic reaction: $2H_2O + 2e^- \rightarrow 2OH^- + H_2$ (2)

Product formation: $Mg^{2+} + 2OH^- \rightarrow Mg(OH)_2$ (3)

Magnesium alloys also have a similar Young's modulus (about 40 MPa) to human bones (about 10-30 MPa), indicating their potential use for implants to treat bone fractures [2]. However, poor corrosion resistance and too rapid degradation, which translates into loss of implant functionality prior to bone fusion, contribute to limiting their widespread use [3].

In order to eliminate the problems a number of works are being undertaken to improve their properties. A widely used solution is the modification of the surface of Mg alloys to produce layers or coatings capable of providing controlled degradation of the implant. One promising method of modifying the surface of magnesium alloys to improve their corrosion resistance is plasma electrolytic oxidation of PEO, which involves the formation of a porous oxide layer [4]. The chemical composition of the produced layer strictly depends on the chemical composition of the bath, and the process parameters used make it possible to obtain coatings of the desired thickness.

Materials and Methods

The test material was WE43 alloy (Goodfellow GMBH, Germany), which in its composition contains magnesium, yttrium, rare earth elements and zirconium. Samples in the form of discs with a diameter of 11 mm and a thickness of 4 mm were ground on SiC sandpaper to a gradation of 1200, wash with ethanol in an ultrasonic cleaner and washed with distilled water. Just before the PEO process, the samples were immersed in a 1% HNO_3 solution for 5 seconds to remove the oxide layer and washed with distilled water.

The plasma electrolytic oxidation process was carried out in an electrolyte containing calcium and phosphorus ions. The PEO was conducted under an impulse current (FIG. 1) up to a fixed voltage with use of a high-voltage power supply PWR 800H.

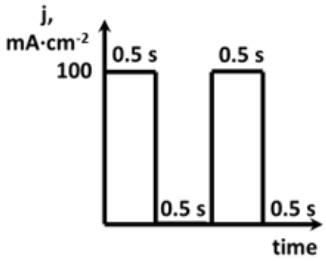

FIG. 1. A schematic illustration of impulse current regime used.

The effect of the produced layer on the properties of the WE43 alloy was then determined. For this purpose, the surface morphology was studied using a scanning electron microscope SEM (TESCAN VEGA) under high vacuum conditions. In addition, EDS analysis (Oxford Instruments Explore) was carried out to identify the chemical composition of the produced layer and the distribution of elements on the surface. Raman spectroscopy was used to determine the chemical compounds on the tested surfaces. Samples of Raman spectra were recorded using a Raman microscope (inVia Renishaw, Gloucestershire, UK) equipped with a charge-coupled device (CCD) detector. Subsequently, corrosion behavior was evaluated using a potentiodynamic method and electrochemical impedance spectroscopy (EIS) study. An Autolab PGSTAT302N potentiostat with Nova 2.1 software was used, the reference electrode, which was a saturated calomel SCE electrode of the KP-113/Ag/AgCl 3M KCl type, an auxiliary electrode in the form of a platinum wire, and the anode, which was the tested sample.

Results and Discussion

Surface observations showed a characteristic porous structure. Chemical composition analyses showed the presence of calcium and phosphorus in the layer formed. Corrosion test results showed that WE43 alloy after PEO treatment had higher values of corrosion potential E_{corr} and polarization resistance R_p, EIS test results confirm better corrosion behavior of the studied magnesium alloy after modification.

Conclusions

The results of the work indicate that by using an electrolyte enriched with calcium and phosphorus elements, it is possible to obtain a good quality protective coating on WE43 alloy, which contributes to improved corrosion resistance. However, there is a need for additional research related to the long-term biodegradation characteristics of the modified material.

Acknowledgements

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COMPOSITES CONTAINING PLANT-ORIGIN CARBON IN EMERGENCY MEDICINE AND WATER RESCUE APPLICATIONS

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Introduction

The current global imperative to mitigate the impact of microplastic pollution on aquatic environments underscores the necessity for adopting biodegradable composites in contemporary water rescue equipment [1]. Traditional materials such as ABS and polycarbonate, commonly utilized in water rescue supplies, are hindered by biocompatibility and weather resistance limitations. This has led to a shift towards more advanced materials, such as activated carbon and plant-origin organic carbon, which offer enhanced mechanical strength and durability [2]. Moreover, these materials align with sustainable development objectives by minimizing environmental impact. Incorporating such innovative materials not only enhances the effectiveness of rescue equipment in challenging conditions but also makes a meaningful contribution towards environmental preservation and the safety of emergency interventions.

Materials and Methods

The preliminary phase of the investigation entailed an in-depth analysis of the raw materials designed for employment in emergency medicine and rescue operations. This involved the incorporation of silica and basalt modifiers and the utilization of FTIR spectroscopy to discern characteristic functional groups. Upon exposure to chlorine, distilled water, and pool water, the samples displayed distinctive FTIR absorption bands with minimal observed influence on material properties. Tribological tests revealed marked disparities in wear characteristics among the samples, particularly underscored by enhanced wear resistance with basalt and increased stiffness with silica modifiers [3]. Subsequent investigations focused on the modification of ABS and PC base materials through the use of active carbon and plant-origin organic carbon. This process involved a meticulous two-stage carbonization procedure to transform green coffee and walnut shells into organic carbon. Initially, the samples experienced reduced pressure and subsequent heating in a drying oven, followed by carbonization in an argon atmosphere at temperatures up to 950°C. Future research is essential to explore composite materials from carbonized walnut shells and green coffee modifiers to develop durable, eco-friendly solutions for rescue operations.

Results and Discussion

The FTIR spectroscopy analysis identified characteristic absorption bands for PLA, silica, and basalt. Upon exposure to chlorine solution, distilled water, and pool water, distinct FTIR bands such as -OH in the 3600-3200 cm^{-1} range and methyl groups in the 3000-2800 cm^{-1} range were observed. Silica-modified samples exhibited additional absorption features corresponding to Si-O bonds, while basalt-modified samples displayed characteristic absorptions related to mineral components such as silicates. These bands exhibited shifts upon exposure to chlorine solution, indicating chemical interactions that affect material properties. Tribological tests using a ball-disk friction node demonstrated significant wear resistance and stiffness variations among the samples. Basalt-modified composites showed enhanced wear resistance under chlorine exposure, while silica contributed to stiffness without significantly affecting hardness. The study illustrated the successful modification of polymers used in 3D printing to achieve desired properties for specific applications. Further analysis involved the modification of ABS and PC with active carbon and plant-origin organic carbon using a powdering technique and standardized homogenization. Filament samples for additive manufacturing were obtained through extrusion under controlled temperatures. For ABS, temperatures were set to 110°C in the loading zone and subsequently to 160°C, 180°C, and 210°C. For PC, temperatures were set to 190°C in the loading zone and then to 240°C, 250°C, and 260°C. Filament with a diameter of 1.75 mm was produced using a linear head and collecting tape. A two-stage carbonization process of green coffee and walnut shells under reduced pressure and an argon atmosphere yielded high-carbon content materials (FIG. 1). Morphological analysis confirmed the successful transformation, highlighting the potential for durable, environmentally friendly materials suitable for emergency medical applications. The results suggest a requirement to create composite material from the modifiers produced after carbonization.

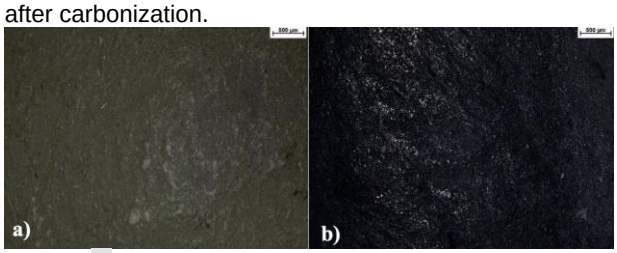

FIG. 1. Microscopy images of a) green coffee bean, b) carbonized green coffee bean.

Conclusions

The initial studies show great potential for advancing the materials used in water rescue and emergency medicine. These findings indicate the possibility of introducing innovative composite materials. This research provides a foundation for further experiments that could develop more durable and functional materials suitable for extreme rescue conditions and less harmful to the environment.

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BIOACTIVE COLLAGEN/BETA GLUCAN MATRICES ENRICHED WITH METHYLGLYOXAL: EVALUATING ANTIMICROBIAL AND BIOCOMPATIBILITY PROPERTIES FOR WOUND CARE

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Introduction

Wound healing is a vital physiological process characterized by a conserved sequence of hemostasis, inflammation, proliferation, and tissue remodeling. A key aspect of this process is the restoration of the epithelial layer, which is essential for regaining the integrity of the skin barrier [1]. Infection can delay wound closure, increasing the disease burden. Wound dressings are crucial in promoting wound healing, particularly those that release antimicrobial agents and offer long-lasting antimicrobial properties [2]. Therefore, the aim of this work was to obtain a material with antimicrobial properties that could actively support wound healing. Based on our previous studies, a mixture of collagen (Coll) and beta-glucan (BG) was used as the matrix. This matrix was enriched with methylglyoxal, an active ingredient derived from Manuka honey, which was supposed to enrich the material with bactericidal properties.

Materials and Methods

The bactericidal activity of a 1% methylglyoxal solution was tested against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. A 1% solution of methylglyoxal in water was used against a bacterial suspension with a density of 10^6 cfu/ml. The bactericidal properties of the solution were assessed at the following time points: T0, T0.25h, T0.5h, T1h, T3h, and T24h. After diluting the samples, 100 µl of the suspension was placed on plates with TSA medium.

A mixture of collagen (1%) and β-glucan (BG) (5%) was prepared in a 90:10 (w/w) ratio. This collagen/β-glucan mixture was modified by adding methylglyoxal at concentrations of 0.125 and 0.25 mg/cm². The materials were prepared using the casting method.

The diffusion of the active component from the matrices was analyzed using the disc diffusion method.

Antibiofilm properties were evaluated using the Lubbock chronic wound model. The dPCR method was employed with primers specific for E. coli, amplifying the uspA gene encoding the universal stress protein and the uidA gene encoding β-D-glucuronidase.

The biocompatibility of the prepared materials was tested against HaCaT cells.

Results and Discussion

The growth of E. coli was inhibited by four orders of magnitude after 15 minutes of contact. After 30 minutes, no colony-forming units were visible on the plate from the first dilution, indicating the amount was below 100 cfu/ml. In contrast, the control sample recorded 2.1 x 10^6 cfu/ml. The growth of S. aureus was slightly inhibited at T1h; however, after 3 hours, no growth of this bacterium was recorded in the test sample, while the control sample recorded 6.4 x 10^6 cfu/ml.

The growth of *P. aeruginosa* was also inhibited after 3 hours of contact with the methylglyoxal bacterial suspension. However, after incorporation into the matrix, the bactericidal properties of methylglyoxal were not as intense. Therefore, higher concentrations of 0.125 mg/cm² and 0.25 mg/cm² were used in the material.

The prepared materials did not inhibit the growth of microorganisms during the diffusion test. Methylglyoxal was not released from the matrix onto the solid medium inoculated with the bacterial suspension.

Antibiofilm properties were studied using the Lubbock chronic wound model, an in vitro model designed to mimic pathogen colonization and biofilm formation in a real chronic wound. Molecular methods are more sensitive than culture methods. Using this method, a decrease in the number of gene copies was observed with an increase in the methylglyoxal content in the material. However, based on the results obtained with the normative method, a better effect was expected.

A key element in the preparation of dressing materials is their biocompatibility with body cells while simultaneously having a biocidal effect. The prepared materials were biocompatible with HaCaT cells. The addition of methylglyoxal to the matrices increased the viability of cells by 7% and 9% compared to the control sample.

Conclusions

The results from Lubbock's method differed from those of other methods, yet they still offer valuable insights. Materials that are bactericidal in standard laboratory tests often do not perform as effectively in conditions that simulate real wound environments. This suggests a need for a more critical approach to testing. Despite the biofilm inhibition being less intense than anticipated, we believe that with suitable drug therapy, the material could be effectively used as an active dressing.

Acknowledgements

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INFLUENCE OF HYDROLYTIC DEGRADATION ON THE MORPHOLOGICAL FEATURES OF TERPOLYMER MATRICES

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Introduction

Poly(lactide-*co*-glycolide) (PLGA) is the most commonly proposed and used biodegradable drug carrier. The release profile of drug substances is dependent on bulk erosion, with the formation and closure of pores predominantly influencing the process heterogeneity [1]. However, poly(trimethylene carbonate) (P(TMC)) is one of the alternatives to PLGA. P(TMC) undergoes surface erosion, which is more homogeneous but also quicker [2]. In this study, poly(L-lactide-*co*-glycolide-*co*-trimethylene carbonate) (P(L-LA:GA:TMC)) was proposed for modification of the degradation process [3].

This study aimed to determine the morphological changes during degradation in non-electron beam (EB) irradiated matrices based on P(L-LA:GA:TMC) with latanoprost (LTP) (P(L-LA:GA:TMC) matrix-LTP) and EB-irradiated matrices with LTP (EB-P(L-LA:GA:TMC)) matrix-LTP)) formulated by the solution casting method. LTP was used as a model liquid drug substance.

Materials and Methods

Matrices (n=50) with LTP based on P(L-LA:GA:TMC) (64.7:17.3:18.0; 59.4 kDa), containing 5% w/w of LTP (Everlight Chemical, Taipei, Taiwan), 10.00 mm ± 0.02 mm in diameter and 0.25 mm ± 0.03 mm thick, were formulated by solution casting at 25°C using 1,1,1,3,3,3-hexafluoro-2-propanol. Twenty-five native matrices were irradiated in an EB accelerator (10 MeV, 360 mA, 25 kGy) (Institute of Nuclear Chemistry and Technology, Warsaw, Poland; certificate no. 625/217/E).

The matrices were incubated in a PBS buffer (pH 7.4, 37°C, 240 rpm).

The morphology changes were observed utilizing a scanning electron microscope (Quanta 250 FEG/FEI, Thermo Fisher Scientific, Waltham, MA, USA) and ImageJ® software, version 1.49e (National Institutes of Health, Bethesda, MD, USA). The following assumptions were made: (i) a circularity value of 1.0 indicates a perfect circle; (ii) a solidity value for the area of a particle divided by its convex hull area indicates the monolithic area, where a value of 1.0 means total solidity; and (iii) heterogeneity signifies the percentage of the elevated area compared to the total area.

Results and Discussion

The degradation period influenced the changes in heterogeneity, circularity, and solidity (TABLE 1).

The heterogeneity increased from 16.5% to 81.7% and from 16.8% to 78.7% for the P(L-LA:GA:TMC) matrix-LTP and the EB-P(L-LA:GA:TMC) matrix-LTP, respectively. No evident pores were observed for either native matrix. However, the changes in the circularity value were observed as a result of the appearance of single pores, sponge-like structures, and oval elements (TABLE 1).

TABLE 1. Morphological parameters of the surface of the matrices during degradation, calculated using ImageJ® software.

P(L-LA:GA:TMC) matrix-LTP				
Time [Days]	**Area [μm²]**	**Heterogeneity [%]**	**Circularity**	**Solidity**
0	4,796.1	16.5	0.039	0.81
1	4,454.8	17.4	0.137	0.82
15	4,739.1	29.7	0.352	0.75
29	4,364.1	28.9	0.349	0.78
43	4,677.2	41.3	0.684	0.51
57	4,673.8	53.3	0.491	0.45
71	4,678.2	58.4	0.414	0.50
85	4,220.9	69.0	0.162	0.31
99	4,772.5	66.1	0.142	0.35
113	4,564.2	81.7	0.652	0.19
EB-P(L-LA:GA:TMC) matrix-LTP				
Time [Days]	**Area [μm²]**	**Heterogeneity [%]**	**Circularity**	**Solidity**
0	4,583.7	16.8	0.036	0.83
1	4,671.8	16.7	0.037	0.83
15	4,096.1	38.8	0.499	0.74
29	4,568.2	38.6	0.416	0.75
43	4,091.4	38.5	0.210	0.75
57	4,527.1	38.7	0.202	0.73
71	4,884.0	63.6	0.152	0.49
85	4,628.5	60.7	0.562	0.43
99	4,844.7	68.5	0.147	0.29
113	4,533.1	78.7	0.719	0.21

Solution casting and EB irradiation did not result in unfavorable features such as disintegration, cracks, microcavities, and slits. For the native P(L-LA:GA:TMC) matrix-LTP and the native EB-P(L-LA:GA:TMC) matrix-LTP, the solidity values were 0.81 and 0.83, respectively. The changes in this parameter during degradation reflect the morphological changes (TABLE 1).

The character of the degradation changes suggests the predominance of surface erosion due to the absence of an evident porous surface. The appearance of single pores may result from EB irradiation.

Conclusions

ImageJ® software is an interesting tool for interpreting degradation changes in biodegradable drug delivery systems.

Acknowledgements

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INFLUENCE OF HYDROLYTIC DEGRADATION ON THE RELEASE PATTERN OF LATANOPROST FROM TERPOLYMER MATRICES

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Introduction

Latanoprost (LTP) is a prostaglandin $F_{2\alpha}$ analog used to lower intraocular pressure in glaucoma treatment, administered daily as eye drops [1]. Generally, for this formulation, poor adherence and low bioavailability are often described [2]. Therefore, in this study, a universal model based on poly(L-lactide-*co*-glycolide-*co*-trimethylene carbonate) (P(L-LA:GA:TMC) with shape memory was proposed for the development of a biodegradable formulation with prolonged release administered intraconjunctivally, intravitreally, subconjunctivally, and subcutaneously. Moreover, the application of a terpolymer with shape memory is particularly important for reducing the invasiveness of administering drug delivery systems, especially by implantation [3]. The application of electron beam (EB) irradiation as a modulating factor for the degradation rate and release pattern was also developed.

Materials and Methods

Matrices (n=50) with LTP based on P(L-LA:GA:TMC) (64.7:17.3:18.0; 59.4 kDa), containing 5% w/w of LTP (Everlight Chemical, Taipei, Taiwan) (P(L-LA:GA:TMC) matrix-LTP), 10.00 mm ± 0.02 mm in diameter and 0.25 mm ± 0.03 mm thick, were formulated by solution casting at 25°C using 1,1,1,3,3,3-hexafluoro-2-propanol. Twenty-five native matrices were irradiated in an EB accelerator (EB-P(L-LA:GA:TMC) matrix-LTP) (10 MeV, 360 mA, 25 kGy) (Institute of Nuclear Chemistry and Technology, Warsaw, Poland; certificate no. 625/217/E).
The matrices were incubated in a PBS buffer (pH 7.4, 37°C, 240 rpm). The following methods and measurements were used: HPLC; NMR; DSC; GPC; water uptake (*WU*); and weight loss (*WL*).

Results and Discussion

LTP was released over 113 days in a tri-phasic model without a burst effect and with a relatively long second release phase, in which changes were observed in glass transition temperature (T_g), molecular weight (M_n), *WU*, and *WL*. EB irradiation decreased the initial M_n, increased *WU*, and accelerated LTP release with a shortened lag phase (FIG. 1, TABLE 1).

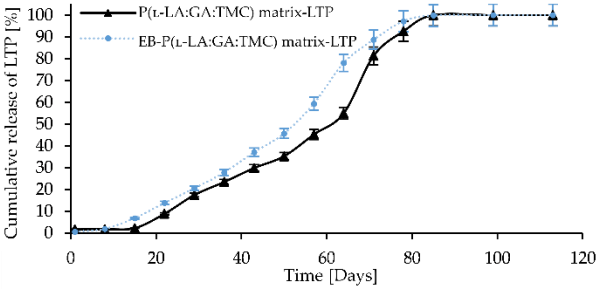

FIG. 1. LTP cumulative release profiles.

TABLE 1. The parameters of the matrices during degradation.

P(L-LA:GA:TMC) matrix-LTP								
Time [Days]	**0**	**15**	**29**	**43**	**57**	**71**	**85**	**99**
F_{LL} [mol%]	62.7	63.1	63.1	62.3	64.5	66.4	65.6	67.8
F_{GG} [mol%]	19.4	18.6	18.6	18.4	16.8	15.6	16.1	15.3
F_{TMC} [mol%]	17.9	18.3	18.3	19.3	18.7	18.0	18.3	16.9
T_m [°C]	ND	ND	ND	81.5	83.8	88.4	94.1	85.5
ΔH [J/g]	ND	ND	ND	8.1	17.0	26.6	31.5	29.6
T_g [°C]	39.9	43.5	42.3	39.4	32.4	33.3	29.6	33.1
M_n [kDa]	42.2	34.3	22.0	12.2	5.0	2.1	1.9	1.9
D	2.061	2.365	2.222	2.313	3.031	1.622	1.537	1.544
WU [%]	0	3.0	3.2	5.6	12.4	46.8	78.2	88.6
WL [%]	0	15.0	16.4	17.2	18.7	49.0	69.7	84.1
EB-P(L-LA:GA:TMC) matrix-LTP								
Time [Days]	**0**	**15**	**29**	**43**	**57**	**71**	**85**	**99**
F_{LL} [mol%]	62.5	63.5	63.5	62.7	63.3	64.9	65.4	70.9
F_{GG} [mol%]	19.7	18.1	18.1	17.9	18.4	18.0	16.3	11.4
F_{TMC} [mol%]	17.8	18.4	18.4	19.4	18.3	17.1	18.3	17.7
T_m [°C]	ND	ND	ND	79.7	80.2	81.1	83.8	92.1
ΔH [J/g]	ND	ND	ND	4.8	12.1	17.7	22.9	44.2
T_g [°C]	43.3	36.0	35.8	34.1	27.8	28.9	30.5	33.9
M_n [kDa]	29.5	20.6	10.3	4.2	2.3	2.2	2.0	1.9
D	2.255	2.384	2.460	2.502	1.804	1.822	1.580	1.534
WU [%]	0	3.2	5.5	35.0	49.2	65.7	78.2	88.3
WL [%]	0	11.7	12.5	15.5	32.1	53.1	71.2	85.7

F_{LL}, F_{GG}, and F_{TMC}—molar percentage of lactidyl, glycolidyl, and carbonate units (respectively) in the terpolymer, T_m—melting temperature, ΔH—melting enthalpy, T_g—glass transition temperature, M_n—molecular weight, *D*—molecular weight distribution, *WU*—water uptake, *WL*—weight loss, *ND*—non-detected.

Conclusions

The proposed model is an interesting solution to develop final medical products providing high adherence.

Acknowledgements

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THE CHARACTERIZATION OF CHITOSAN/PHENOLIC COMPOUNDS SCAFFOLDS

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Introduction

Chitosan is a natural polysaccharide and has antibacterial activity, along with antifungal, mucoadhesive, analgesic, and haemostatic properties. It can be biodegraded into non-toxic residues and is noncytotoxic [1]. Phenolic acids are natural compounds proposed as effective cross-linkers for biopolymers [2]. The aim of the study was to compare the properties of scaffolds obtained from chitosan modified by gallic acid, ferulic acid, and tannic acid for the application in tissue engineering.

Materials and Methods

Chitosan (CTS; shrimp sourced, low molecular weight, Mv=375 kDa, DD=76.46 ± 0.22%), tannic acid (TA), gallic acid (GA), and ferulic acid (FA) were dissolved in 0.1M acetic acid at 1% concentration separately. Chitosan solution was mixed with 10 (w/w%) addition of phenolic acid solution for 1 h. The mixtures were then placed in the 24-well sterile cell culture plates (2.5 mL/well), frozen for 24 h in -18°C, and lyophilized (−20°C, 100 Pa, 48 h, ALPHA 1–2 LDplus, CHRIST, Berlin, Germany), −20°C, 100 Pa, 48 h). As a result, the three-dimensional dry samples (scaffolds) were obtained. The blood compatibility was studied according to procedure [3]. 0.2 mL of anticoagulated (by citrate phosphate dextrose adenine addition) sheep blood was added to 10 mL of physiological saline solution containing different specimens. After 1 h, the suspension was centrifuged (1000 rpm, 10 min) and absorbance of the supernatant of each tube was measured at 540 nm by microplate reader Multiscan FC (Thermo Fisher Scientific, Waltham, USA). An in vivo experiment was conducted on four male New Zealand rabbits (2.8-3.2 kg) from the Medical University of Silesia in Katowice. The rabbits were vaccinated and monitored in accordance with European Directive 2010/63/EU and the Local Ethics Committee of the University of Life Sciences in Lublin. After acclimatization, the rabbits underwent surgery for the implantation of chitosan scaffolds modified with gallic acid (CTS_GA), ferulic acid (CTS_FA), and tannic acid (CTS_TA), as well as control scaffolds (CTS). Post-surgery care included antibiotics and anti-inflammatory drugs. Mild swelling was observed, but the rabbits remained healthy. Three months later, the rabbits were euthanized, and tissue samples from the implantation sites were collected for analysis.

Results and Discussion

According to the ASTM F756-00 standard, materials with a hemolytic index between 0-2% are classified as non-hemolytic, while materials with 2-5% are slight hemolytic and ><5% are classified as hemolytic [4]. The results of blood compatibility measurement are listed in TABLE 1. The hemolysis for all tested scaffolds is below 2%. It suggests that materials are nonhemolytic. In the control sample (FIG. 1A), fragments of fibrous material (scaffold) were observed within the subcutaneous adipose tissue, surrounded by moderate infiltration of lymphocytes, macrophages, and multinucleated giant cells, along with connective tissue proliferation. In the CTS_GA sample (FIG. 1B), no implant was observed, but focal proliferation of cell-poor, collagen-rich connective tissue and atrophy of skin adnexa indicated scaffold resorption and replacement by connective tissue, suggesting tissue healing and repair. In the CTS_FA sample (FIG. 1C), minimal resorption of the implant was noted. The large implant remained in the subcutaneous tissue, surrounded by macrophages, multinucleated giant cells, neutrophils, and lymphocytes, with peripheral connective tissue proliferation. In the CTS_TA sample (FIG. 1D), no implant was visible. Slight cutaneous fibrosis and adnexa atrophy were observed, though not grossly discernible.

TABLE 1. The hemolysis rate for all tested samples (n= 5; * significantly different from CTS — $p < 0.05$).

Specimen	Hemolysis rate [%]
CTS	1.59 ± 0.21
CTS_GA	1.12 ± 0.17*
CTS_FA	0.98 ± 0.15*
CTS_TA	1.74 ± 0.10

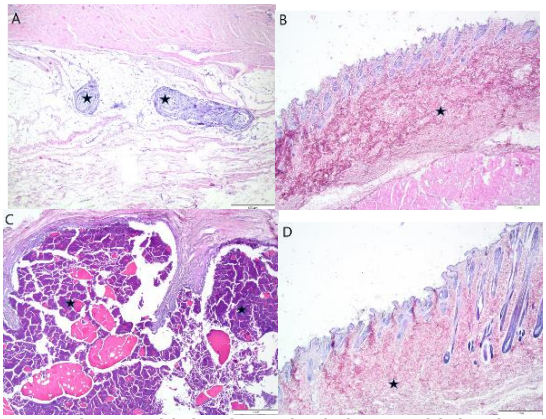

FIG. 1. Tissue with implanted: A) tissue with implanted chitosan scaffold (asterisks -fragments of scaffolds, B) chitosan/gallic acid scaffold (asterisk - collagen-rich connective tissue), C) chitosan/ferulic acid (asterisks – fragments of scaffold), D) chitosan/tannic acid (asterisk – subsequent cutaneous fibrosis).

Conclusions

All scaffolds showed a hemolysis rate below 2%, indicating they are safe for blood contact. Histological images of rabbit subcutaneous tissue implants revealed faster resorption and tissue organization for scaffolds modified with gallic and tannic acids compared to ferulic acid. All tested materials are biocompatible and safe for medical use.

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STRONTIUM TITANATE-ENHANCED PVA FILMS FOR ADVANCED WOUND CARE APPLICATIONS

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Introduction

Skin, the largest organ of the human body, performs vital functions like physical barrier maintenance, thermoregulation, and water loss regulation. Wound dressings are crucial in accelerating wound healing, a field of significant interest. Polyvinyl alcohol (PVA), known for its biocompatibility and film-forming properties [1], is widely used in medical applications such as wound dressings [2]. Strontium Titanate (SrTiO3) nanoparticles, known for their dielectric properties and biocompatibility [3], offer potential enhancements to PVA-based films. This study aims to synthesize and evaluate novel PVA films impregnated with SrTiO3 nanoparticles for their mechanical properties, biocompatibility, and antibacterial activity.

Materials and Methods

PVA in granular form and $SrTiO_3$ nanoparticles were procured from Merck. A 6%wt PVA stock solution was prepared by dissolving PVA granules in distilled water at 90°C. $SrTiO_3$ nanoparticles were added to the PVA solution in varying concentrations (0.5%-20%wt) and stirred at 60°C for 5 hours. The mixtures were poured into Petri dishes and left to dry at room temperature. Mechanical properties were assessed using a Shimadzu EZ-Test machine, while surface roughness was analyzed using a NanoScopeMultiMode microscope. Water content was measured by drying samples at 105°C. Data were analyzed using SigmaPlot 14.0 (Systat Software, San Jose, CA, USA). Normality was assessed with the Shapiro–Wilk test. Results were presented as mean ± standard deviation (SD) and analyzed using one-way ANOVA. Multiple comparisons against the control group were performed using the Bonferroni t-test, with statistical significance set at $p < 0.05$.

Results and Discussion

The mechanical properties indicated that adding $SrTiO_3$ decreased film stiffness but increased tensile strength at higher concentrations (FIG. 1). Surface roughness measurements showed increased roughness with higher nanoparticle content, suggesting improved cell interactions (TABLE 1). Water content analysis revealed higher water retention with increased $SrTiO_3$, beneficial for wound healing. These findings align with previous studies highlighting the positive effects of nanoparticles on polymer film properties.

TABLE 1. Roughness parameters (Ra and Rq) and the water content in films based PVA with and without STO (grams of water per 100 g of a dry sample, n = 5; * significantly different from PVA—$p < 0.05$)

Specimen	Ra [nm]	Rq [nm]	Water Content [g/100g]
PVA	1.75 ± 0.09	2.22 ± 0.04	3.72 ± 1.05
PVA + 5%STO	1.83 ± 0.06	2.27 ± 0.03	6.09 ± 0.91*
PVA + 10%STO	2.22 ± 0.02*	2.80 ± 0.07*	7.25 ± 0.77*
PVA + 20%STO	2.47 ± 0.03*	3.25 ± 0.02*	9.17 ± 1.17*

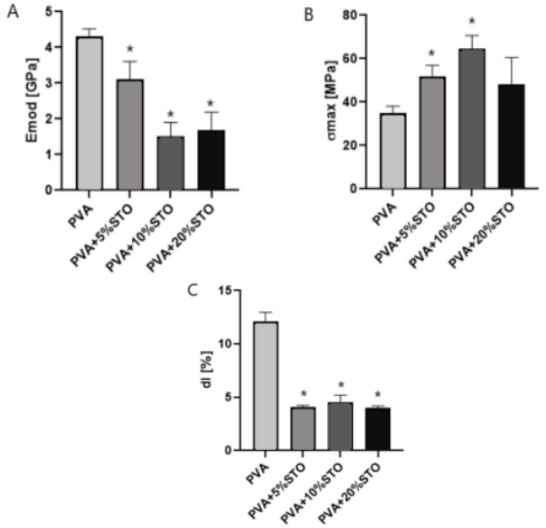

FIG. 1. A) the Young Modulus (E_{mod}), (B) maximum tensile strength (σ_{max}) and (C) elongation at break (dl) determined for films based on PVA, PVA + 5%STO, PVA + 10%STO, and PVA + 20%STO. *significantly different from PVA ($p<0,05$).

Conclusions

This study demonstrates that PVA films incorporated with $SrTiO_3$ nanoparticles exhibit enhanced mechanical properties, biocompatibility, and antibacterial activity. The modifications improved surface roughness and water retention, making the films suitable for wound dressings and other biomedical applications. Future research will focus on further characterizing the bioactive effects on skin regeneration and optimizing degradation rates. These PVA/$SrTiO_3$ films hold potential as bioactive dressings, implant coatings, and packaging materials.

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THE BIOLOGICAL PROPERTIES OF STRONTIUM TITANATE-ENHANCED PVA FILMS

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Introduction

The skin, as the body's largest organ, serves as a protective barrier, regulates temperature, and controls water loss. When damaged, effective healing is crucial to prevent infections and promote repair. Wound dressings aid this process, and researchers aim to develop optimal materials. Polyvinyl alcohol (PVA) stands out due to biodegradability, biocompatibility, and film-forming properties [1]. These properties make PVA suitable for medical applications such as wound dressings [2]. This study explores PVA films with strontium titanate (STO) nanoparticles, known for their dielectric and flexoelectric properties [3], to enhance wound dressing performance.

Materials and Methods

PVA (MW 31,000–50,000, 98–99% hydrolyzed) and STO nanoparticles (~100 nm) were used to prepare PVA/STO films. A 6%wt PVA solution was mixed with varying amounts of STO (0.5%wt to 20.0%wt), stirred, and cast into films. In vitro hemo- and cyto-compatibility tests were conducted using human red blood cells (RBCs) and an osteoblast cell line (hFOB 1.19), evaluating cell viability through MTT and LDH assays. The biocidal properties of PVA/STO films were evaluated using ISO 22196 with *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. Statistical analysis was performed using one-way ANOVA and the Bonferroni t-test.

Results and Discussion

In vitro tests indicated that PVA/STO films did not exhibit hemolytic effects (FIG. 1) and were non-toxic to RBCs and osteoblasts for up to 24 h (FIG. 2a). However, longer incubation (72 h) (FIG. 2b) revealed a slight cytostatic effect at higher STO concentrations. The enhanced biocidal activity against Gram-positive and Gram-negative bacteria suggests potential applications in wound healing. PVA films with STO showed strong antibacterial properties, especially at higher concentrations, with no significant differences between strains (FIG. 3). The flexoelectric properties of STO nanoparticles further support their use in biomedical applications, promoting cell growth and tissue regeneration.

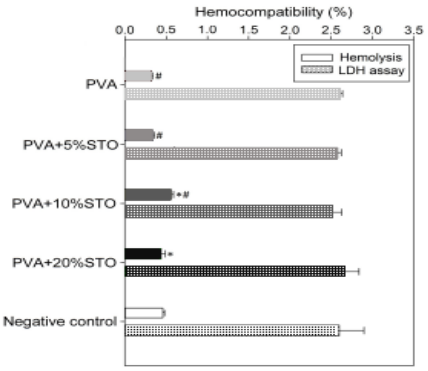

FIG. 1. The effect of developed films on hemo-compatibility of human erythrocytes (hemolysis rate and lactate dehydrogenase release) after 24 h exposure to films (n = 4, data are expressed as the mean ± SD, * significantly different from the negative control and # significantly different from the PVA ($p < 0.05$)).

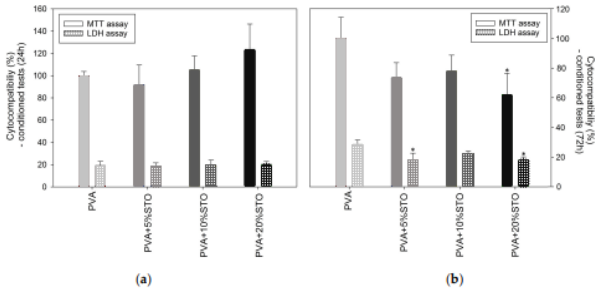

FIG. 2. The effect of developed films on cyto-compatibility of hFOB 1.19 cells (cell viability and lactate dehydrogenase release): (a) after 24 h and (b) after 72 h exposure to films extracts (n = 4; data are expressed as the mean ± SD, * statistical significance compared to the control–PVA ($p < 0.05$)).

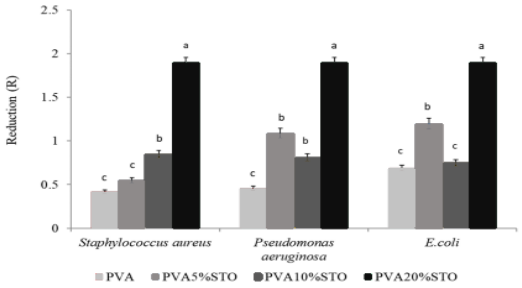

FIG. 3. Biological properties of PVA/STO films. Different letters over the bars indicate a significant difference between means ($p < 0.05$)

Conclusions

The study demonstrates that PVA films incorporating STO nanoparticles possess favorable properties for wound dressing applications, including enhanced stability, biocompatibility, and antibacterial activity. These findings suggest that PVA/STO composites can be considered as potential materials for advanced wound dressings, promoting effective healing while maintaining biocompatibility. Further research is necessary to optimize the films' properties and evaluate their performance in clinical settings.

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DEVELOPMENT OF A HYDROGEL TO PREVENTING SKIN DAMAGE – PRELIMINARY STUDIES

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Introduction

Abrasions are common superficial skin injuries that typically heal quickly but can become serious if not properly treated. While minor abrasions usually heal within a week without scarring, more extensive ones can take longer and may bleed and scar. Ignoring abrasions and simply applying an adhesive bandage can lead to inflammation and infection, characterized by pain, swelling, and pus, which in severe cases, can be fatal.
Common causes of abrasions include wearing new or ill-fitting shoes, leading to chafing, blisters, and eventually painful wounds. Similarly, clothing made from artificial fabrics and excessive sweating, especially in hot and humid conditions, can cause skin abrasions. These issues are exacerbated in obese individuals due to skin folds and moisture accumulation, and in athletes exposed to extreme conditions.
Preventing abrasions is preferable, but current preventive measures and products are limited. Many products lack protection from external factors, leading to oxidation and loss of active ingredients' beneficial properties. Encapsulation of active ingredients can preserve their properties, extending their efficacy.
The project's goal is to develop a hydrogel formulation enriched with encapsulated active ingredients. This biopolymer-based hydrogel will moisturize and lubricate the skin, preventing abrasions and accelerating healing of micro-injuries with its anti-inflammatory properties.
Encapsulation will increase the stability and effectiveness of the active ingredients, providing a more reliable and long-lasting wound healing solution. This innovative approach aims to improve skin protection and enhance the healing process, addressing the limitations of current wound care products.

Materials and Methods

The work plan involved several steps. Firstly, it included the selection of suitable polymers for the capsule shells, such as sodium alginate and chitosan, ensuring compatibility with *Centella asiatica* oil. Secondly, it focused on developing an encapsulation technique and optimizing parameters to achieve stable microcapsules. Thirdly, the plan involved creating a methodology to produce a hydrogel with appropriate physicochemical properties, selecting preservatives, thickeners, and other ingredients to ensure the even suspension of microcapsules in the formulation mass.

Results and Discussion

The primary aim of this project was to develop and preliminarily assess a dermatological product designed to prevent skin abrasions while offering prolonged hydration, lubrication, and soothing effects. The project's outcome is a hydrogel formulation infused with active ingredients like panthenol, glycerin, royal jelly, and encapsulated *Centella asiatica* oil. *Centella asiatica* oil enhances skin firmness, elasticity, and hydration, and has regenerative, anti-inflammatory, and soothing properties, making it beneficial for treating atopic lesions and psoriasis. Panthenol is known for its moisturizing, anti-inflammatory, and epidermal regeneration acceleration properties. Glycerin serves as a moisturizer and penetration promoter, while royal jelly has antibacterial properties, promotes collagen production, and enhances skin smoothness and firmness. These components will ensure comprehensive skin care, prevent epidermal damage, and soothe existing lesions. The hydrogel will form a protective film, keeping the skin moisturized and lubricated, thereby reducing friction and discomfort from itchy and burning skin. As a result of the research, a stable preparation was obtained, which will be studied further in terms of application properties.

Conclusions

The goal of the work was achieved, resulting in a stable preparation that, due to the selected ingredients, can potentially be used in formulations to protect the skin against damage. However, the final formulation still requires optimization and testing of its physicochemical and application properties with the participation of probands.

Acknowledgements

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DEVELOPMENT OF PVA-BASED HYDROGEL MASKS IMPREGNATED WITH ASTAXANTHIN

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Introduction

The development of hydrogel materials facilitates the creation of multifunctional face masks that offer skin care benefits and support the lipid barrier [1]. Their superior moisturizing properties are attributed to the three-dimensional polymer networks that retain high water content [2]. An advantageous base for hydrogel masks is Poly(vinyl alcohol) (PVA), a safe polymer that ensures biocompatibility and excellent barrier properties [3]. To further enhance and stabilize the coating's properties, active compounds like astaxanthin can be incorporated. Astaxanthin, is a xanthophyll carotenoid found in *Haematococcus pluvialis Chlorococcum, Phaffia rhodozyma*, and *Chlorella zofingiensis* [4], exhibits antioxidant and anti-inflammatory effects, benefiting inflammatory conditions and oxidative stress. The broad functional spectrum of astaxanthin in combating various diseases makes it a suitable multifunctional pharmacological agent [5].

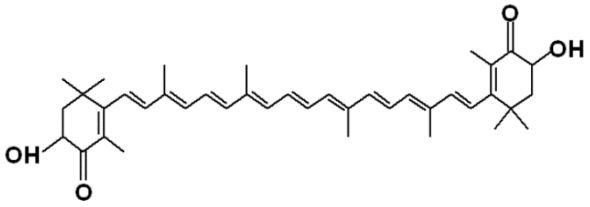

FIG. 1. Planner structure of Astaxanthin [4].

Materials and Methods

The synthesis of hydrogel masks was conducted using a UV lamp, which allowed for the formation of the desired structure in any shape. PVA was used as the coating base and the matrix was enriched with astaxanthin. Fourier-transform infrared spectroscopy (FTIR) was performed to verify the composition's purity. Incubation tests were conducted in fluids simulating the body's environment like SBF to assess sorption capacity and analyze pH changes. Finally, the surface morphology of the masks was examined using scanning electron microscopy (SEM).

Results and Discussion

The selected composition allows for the creation of PVA-based hydrogel masks impregnated with astaxanthin, as confirmed by extensive syntheses aimed at evaluating specific physicochemical properties. The individual reagents significantly impacted the results achieved. The mask demonstrates sorption capacity, which is crucial for its potential future applications. Additionally, its composition is pure and contains the appropriate functional groups. Incubation studies confirm biocompatibility and non-toxicity. The material did not degrade in the incubation fluids. Surface morphology analysis confirmed the formation of a properly cross-linked hydrogel structure. Astaxanthin positively influenced the final outcome of the obtained hydrogel mask.

Conclusions

PVA-based hydrogel masks, enriched with active compounds such as astaxanthin, can be utilized as carriers for therapeutic substances for external skin applications. They provide optimal moisturizing conditions and support skin regeneration. The future development of such medical materials appears very promising, and further research in this area will enhance the quality of dermatological solutions and contribute to improving the quality of life, particularly for individuals with skin problems.

Acknowledgements

The research work was carried out within the SMART-MAT Functional Materials Science Club, BioMat section at the Faculty of Materials Engineering and Physics of the Cracow University of Technology within the framework of "Hybrid materials to for stimulating collagen production in the skin" SKN/SP/602130/2024.

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HYDROGEL MASKS BASED ON HYALURONIC ACID/ CHONDROITIN SULFATE PREPARED BY UV IRRADIATION TECHNIQUE

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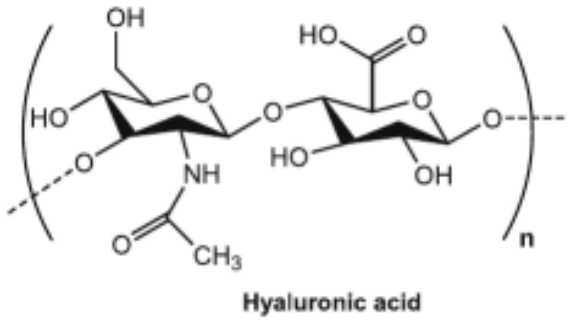
Introduction

Hydrogel masks enriched with hyaluronic acid and chondroitin sulphate represent a promising strategy for the transdermal delivery of active ingredients to the skin. It is a substance naturally occurring in the human body. Hyaluronic acid is a major component of the extracellular matrix [1,2]. This substance is known for its exceptional water binding capacity, providing intensive hydration [3]. Chondroitin sulphate occurs naturally in joint cartilage, lungs, skeletal muscles and skin. It plays an important role in maintaining the structure and function of these tissues [4]. These two ingredients in combination improve the skin's hydration, support the production of collagen and elastin, which improves the skin's elasticity, provide a protective layer for the skin against pollution and UV radiation, have an anti-wrinkle effect [5].



Hyaluronic acid

FIG. 1. Chemical structure of hyaluronic acid [6].

Materials and Methods

The hydrogel masks were created by means of synthesis under the influence of a UV lamp. Hyaluronic acid and chondroitin sulphate were used as the coating base. To check the purity of the composition of the masks, Fourier transform infrared spectroscopy was performed (FTIR). To assess their sorption capacity and study pH changes, incubation tests were performed in Simulated body fluid (SBF), which simulates human body fluids. Finally, scanning electron microscopy (SEM) was used to examine the surface morphology of the masks.

Results and Discussion

The developed formulation allows the creation of hyaluronic acid/chondroitin sulphate-based hydrogel masks using a UV irradiation method. The appropriate choice of chemical composition quite significantly affects the physicochemical properties of the material formed. The masks have sorptive capacities that play a significant role in their medical application. FTIR showed that the matrices obtained have a pure composition and the corresponding functional groups. The study confirmed that the material is biocompatible and non-toxic, and did not degrade in SBF fluid. Analysis by SEM confirmed the corresponding hydrogel structure.

Conclusions

Hydrogel masks based on hyaluronic acid and chondroitin sulphate can serve as carriers of therapeutic substances for application to the skin. The resulting masks improve skin hydration and promote skin regeneration. Hyaluronic acid in combination with chondroitin sulphate can do a lot of good in dermatological applications. Given the current emphasis on skin care, especially complexion care, the development of this material is limitless.

Acknowledgements

The research work was carried out within the SMART-MAT Functional Materials Science Club, BioMat section at the Faculty of Materials Engineering and Physics of the Cracow University of Technology within the framework of "Hybrid materials to for stimulating collagen production in the skin" SKN/SP/602130/2024.

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POLYMER-PEPTIDE COSMETIC MASK MODIFIED WITH ZINC OXIDE NANOPARTICLES TO PROMOTE SKIN REGENERATION DURING ACNE VULGARIS TREATMENT

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Introduction

The skin is one of the largest organs of the human body, the first barrier that protects the body from external factors. Unfortunately, this also makes it extremely vulnerable to burns and the formation of wounds resulting from mechanical trauma. The process of wound healing is complex, and in addition, disruption of this process leads to paralysis of part of the tissue mass, resulting in a non-functional part, commonly referred to as a scar [1]. Undesirable phenomena that interfere with the normal functioning of the skin are disorders. One of the most commonly reported conditions in adolescents as well as in mature individuals is acne. The disease is characterised by excessive sebum production, leading to seborrhoea and subsequent inflammation. One of the causes of the problem is the bacterium Cutibacterium acnes, which has been reported to develop antibiotic resistance [2]. Another factor leading to acne is stress. Experienced uninterruptedly over a long period, it leads to oxidative stress, resulting in the appearance of free radicals that destroy collagen-elastin fibres.
The way to solve the acne problem is to use the right combination of biomaterials. Zinc particles play a huge role in the proper functioning of the body. They functions as a cofactor in transcription factors, responsible for proper metabolism and wound healing [2]. Nano zinc ions have strong antibacterial, anti-inflammatory properties and accelerate skin tissue regeneration [3]. A widely used protein in treatment and supplementation is glutathione. Glutathione is a natural tripeptide produced by the human body, whose most important characteristic is its antioxidant property [4]. A promising solution to the problem of acne is the combination of zinc nanoparticles with glutathione, in the form of a hydrogel matrix, which will provide efficacy in the fight against acne along with comfort.

Materials and Methods

In the present study, a hydrogel mask was obtained by combining polyethylene glycol 6000, glutathione, sodium alginate and the cross-linking agent poly(ethylene glycol) diacrylate. In addition, zinc oxide nanoparticles were synthesised using zinc acetate and sodium hydroxide to modify the biomaterial. The polymerisation process was performed under UV light.

Fourier-transform infrared specroscopy (FT-IR) was carried out to analyse the chemical composition of the zinc nanoparticles and the prepared biomaterial. The morphology and chemical composition of the resulting zinc nanoparticle powder was examined using Scanning Electron Microscopy (SEM) with microanalysis of composition (EDS). In addition, a qualitative release study of the zinc nanoparticles was performed through UV-Vis spectroscopy. The effect of the obtained biomaterial on changes in pH values, conductivity and sorption capacity in the environment of Ringer's fluid was also investigated.

Results and Discussion

Examination of the composition of the samples by FT- IR shows characteristic peaks from the functional groups of the individual pure components in the matrix.
Furthermore, FT-IR and EDS microanalysis confirm the successful synthesis of zinc nanoparticles. UV-Vis examination shows the presence of nanoparticles in the biomaterial during release in artificial incubation solutions. In addition, the selected material composition reduces the pH value.

Conclusions

In conclusion, based on the results obtained during synthesis under a UV light, a flexible hydrogel matrix was obtained. The results indicate that a suitable chemical composition of the products was obtained. In addition, the biomaterial in the artificial environment of body fluids has a good sorption capacity, is able to effectively change the pH value and is also able to release zinc nanoparticles. The hydrogel biomaterial obtained in this way can find application in skin support during the treatment of acne vulgaris.

Acknowledgements

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DEVELOPMENT OF HEMOCOMPATIBILE ALD COATINGS FOR MATERIALS DEDICATED FOR THE IMPLANTS IN THE CARDIOVASCULAR ENVIRONMENT

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Introduction

An atrial septal defect (ASD) is one of the most common congenital heart defects in children and the most frequent congenital defect found in adults [1]. Currently, several types of kits are available for percutaneous closure of ASD [2]. The latest research is seeking ways to improve the biocompatibility of NiTi-based implants due to the high nickel content and the risk of nickel ion release (corrosion in the body environment). The first is to replace the nickel in the alloy with less toxic elements such as Pt, Pd, Zr, Hf, or Nb, but alloy additives significantly change the characteristic temperatures of the martensitic transformation, excluding the possibility of using these alloys in medicine. Another way to improve the hemocompatibility of NiTi alloys is to modify their surface [3].

Materials and Methods

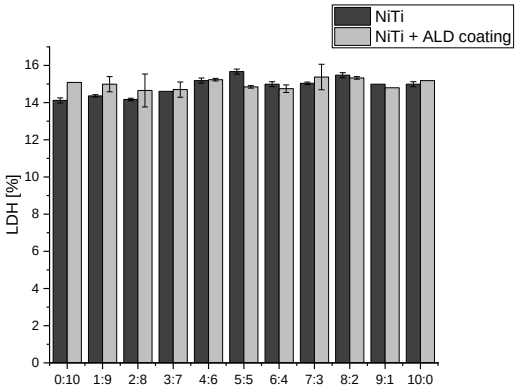
As part of this work, the conditions for the production of TiO_2 surface layers using the ALD method and laser surface modification were developed. The aim was to develop coatings and correlate the new surfaces, which should reflect the structure of the parent material (NiTi alloy), with their biocompatibility and hemocompatibility. Biocompatibility was determined by direct cytotoxicity tests and on extracts. The level of LDH (lactate dehydrogenase) secreted from the tested samples was analysed. In terms of coagulation system activation on the produced samples with the layers, the process of protein adsorption and surface protein desorption was analysed. For the process of surface protein desorption and biofilm formation on the surface of the samples, FBS (fetal bovine serum) (Sigma Aldrich) and whole human blood were used. The aging process in blood over time was analysed for changes in coagulation system activation. In the next step of the research, the focus was on the blood-material interaction. The tests were conducted on:

- NiTi alloy samples with different surface modifications (ALD, laser nanostructuring).
- Comparative control samples.

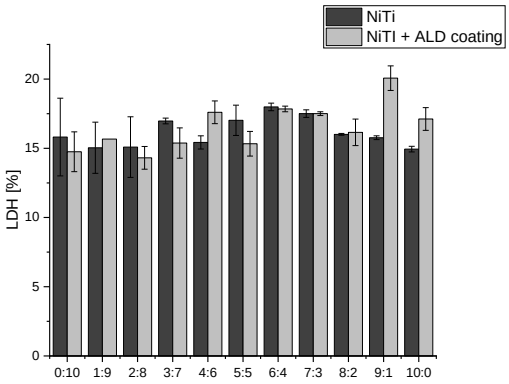
This comprehensive analysis aimed to understand the interaction between blood and the material, focusing on the effectiveness of the surface modifications in enhancing biocompatibility and reducing the risk of thrombosis and other complications. Blood-surface interactions in dynamic conditions were assessed on the new device self-elaborated high speed rotor adapted to the conditions found in the flow of blood. The tested materials, after carrying out appropriate tests, were prepared for the assessment of surface coverage by morphotic blood elements using confocal laser scanning microscopy.

Results and Discussion

Based on the results of the LDH analysis (FIG. 1), a similar level of LDH was observed for subsequent samples, both for 24 h and 48 h. An increase in the LDH level was noted for the 9:1, 48h sample (NiTi surface + coating).



a)



b)

FIG. 1. Analyses of LDH after: a) 24 h incubation; b) 48 h incubation.

Analysis of samples after cytotoxicity tests using the direct method with confocal microscopy images allowed for the observation of a low level of cytotoxicity and a similar number of cells for both the reference material and the test material modified with TiO_2. Based on the analyses using CLSM, no activation of the coagulation system was observed in tests using blood under dynamic conditions.

Conclusions

Based on the results obtained, no toxic effects were observed from the material subjected to surface treatment using the ALD method and laser nanostructuring. The results from the surface passivation test using protein and preliminary hemocompatibility studies of the samples showed good material properties in the context of blood-material interactions. The new material could be successfully used in the human circulatory system as an implant.

Acknowledgements

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MICROBIOLOGICAL ASSESSMENT OF BIOMATERIALS DEDICATED TO SURGICAL INSTRUMENTS

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Introduction

Hospital-acquired infections and growing antibiotic resistance of bacteria constitute a significant problem of modern medicine [1]. One approach is to minimize the risk of fatal infection is to use antimicrobial coatings on surgical instruments. The work concerns the microbiological assessment of the risk of bacterial biofilm formation on materials intended for tools dedicated to blood vessel surgery, in particular the flow closure clamp [2]. The core area of our innovations is cardiovascular surgery. State-of-the-art cardiovascular and thoracic clamping and gripping instruments (CVTCGI) are not devices with real-time pressure force control. The paper presents a correlative analysis of the interaction between bacteria and the surface of a material with a purpose for surgical instruments.

Materials and Methods

In this study two types of biomaterials were tested: 80A and 50A, that were subjected to two types of sterilization: autoclave (Steam) and ethylene oxide (EO). Main objective was to evaluate the impact of sterilization on the surface susceptibility to bacterial colonization. In order to achieve this, Gram-negative *Escherichia coli* ATCC 8739 and Gram-positive *Staphylococcus aureus* 6538P were used. Bacteria were incubated on the surface of tested materials for 7 days at 37ºC and 80% humidity using nutrient broth for *E. coli* and tryptic-soy broth for *S. aureus* as a medium. After incubation, samples were washed three times (3 x 5 min, 120 rpm) using phosphate buffered saline (PBS, pH = 7.4) and fixed by incubating in 4% formaldehyde solution at 4ºC. Then, samples were washed again as described above and fluorescence in situ hybridization (FISH) staining was performed. For *E. coli*, Cyanine 3 labeled DNA probe (pB-3938) [3] was used and *S. aureus* probe (pB-349) [3] was labelled with Cyanine 5. Both probes were synthetised by Biomers (Germany). The FISH staining was analysed using confocal laser scanning microscopy (CLSM) Exciter 5 Carl (Zeiss, Germany) equipped with LD EC Epiplan Neofluar 50x (Carl Zeiss, Germany) in order to detect bacterial biofilm formation. The scanning confocal microscopy technique was verified by analysis using a SCIOS II scanning electron microscope (ThermoFisher, USA), equipped with a FEG (Field Emission Gun) electron gun (30 kV). Bacteria-surface interaction studies were analyzed using low, 5kV accelerating voltage in order not to disrupt the biofilm structure. Prior SEM analysis, samples were dehydrated coated with an Au/Pd conductive layer.

Results and Discussion

On the surface on the 80A material, more *S. aureus* bacterial cells on the autoclaved surface is observed, the bacteria form large clusters and there are large uncolonized spaces between the clusters. On the contrary, observations in the case of the 50A are the opposite - there are more *S. aureus* bacterial cells on the surface previously sterilized with ethylene oxide, moreover, the bacteria are more evenly distributed on the surface in smaller clusters, there are no larger clusters and spaces free from colonization. For 80A material there are no noticeable differences between the number of *E. coli* cells for regardless of sterilization method, moreover there are noticeable uncolonized spaces. In the case of 50A, there are slightly more *E.coli* cells on the surface previously sterilized with ethylene oxide, uncolonized spaces are also noticeable.

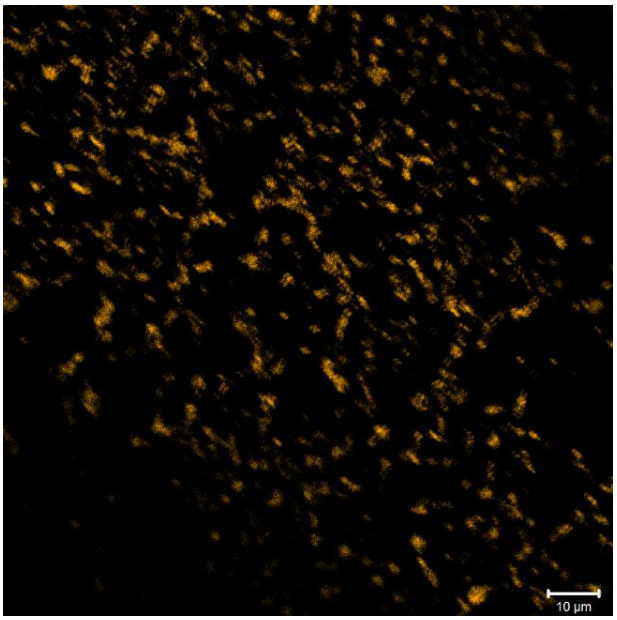

FIG. 1. FISH staining of 50A material. Orange (Cy3) – *Escherichia coli* cells on the surface.

Conclusions

The sterilization method affected colonization by bacteria, this should be taken into account when choosing the sterilization method. Generally, the 80A material was less susceptible to the method of sterilization compared to 50A.

Acknowledgements

The project was financed by the National Center for Research and Development granted (NCBR) and Österreichische Forschungsförderungsgesellschaft (FFG) under the M-ERA.NET3/2022/75/AtraumaBioMat/2023 and 900377 of M-ERA.NET 3 Call 2022. Some of the tasks were carried out as part of the statutory work under task Z-8

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BIOFUNCTIONALIZATION THROUGH THE USE OF POLYELECTROLYTE MICELLES AND ERYTHROCYTES

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Introduction

A cell's response to a material is a complex process that is crucial for understanding many biological and medical phenomena. Modification with polyelectrolytes allows for changes in surface characteristics and tailoring them to the desired outcomes [1,2]. Polyelectrolytes, with their unique chemical properties, enable precise control over the surface properties of materials, such as hydrophilicity, surface charge, and biocompatibility [3]. By using different types of polyelectrolytes and application techniques, the surface of materials can be tailored to specific requirements, which is of crucial. In this study, the response of cells to contact with polyelectrolytes in the form of layers, micelles, and complex polyelectrolyte systems with erythrocytes as carriers of growth factors was investigated [4].

Materials and Methods

Polyelectrolytes were applied by the Layer-by-Layer method, utilizing a robot that sequentially immerses the samples in solutions to deposit the layers (FIG. 1).

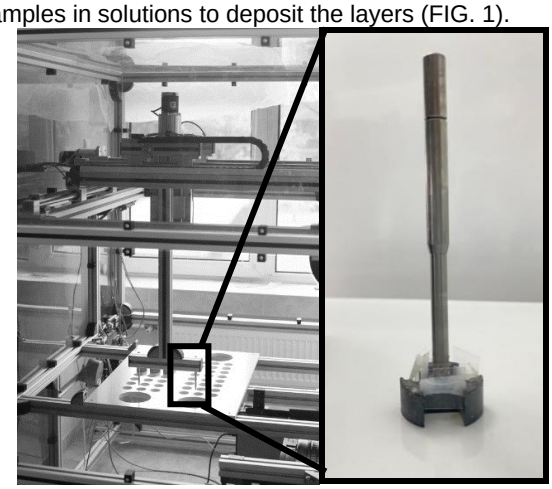

FIG. 1. Robot designed for applying polyelectrolyte layers along with a holder containing the samples.

Depending on modification, polycations were a solution of chitosan or chitosan grafted with poly(N-isopropylacrylamide (PNIAPAM) with a concentration of 0.5 mg/ml and the polyanions were a chondroitin sulfate or grafted sodium alginate with final concentration of 1 mg/ml. For one modification, erythrocytes were used as a growth factors delivery system. The erythrocyte concentrate was obtained from the Regional Blood Donation and Blood Treatment Center (RCKiK). For each modification, 12 bilayers were applied, and three replicates were prepared. The samples prepared in this manner were subjected to antibiotic treatment to ensure sterility. Human Umbilical Vein Endothelial Cells (HUVEC) were used along with a complete medium and supplements provided by the producent. The cells were incubated on the samples for 72 hours. After this time, the cells were fixed with 4% paraformaldehyde and stained. All analyses were conducted using a confocal laser scanning microscope Carl Zeiss LSM 5 Exciter.

Results and Discussion

The current study was to verify the layers using HUVEC. These studies were aimed at defining the monolayer. Progenitor cells were used to determine the level of i-Cell differentiation to HUVEC. The cells stained with selected dyes are shown in FIG. 2. In FIG. 2A, the leukocyte-endothelial cell adhesion molecule E-selectin is visible, while in FIG. 2B, the platelet-endothelial cell adhesion molecule can be observed. These two dyes indicate the degree of differentiation of the applied cells. The greater the number of visible structures stained with these dyes, the better, as it indicates an advanced differentiation process. In FIG. 2C, tight junctions between cells can be observed. The continuous lines formed between cells indicate the formation of a properly developed cell monolayer. In FIG. 2D, cell nuclei were shown to determine cell count.

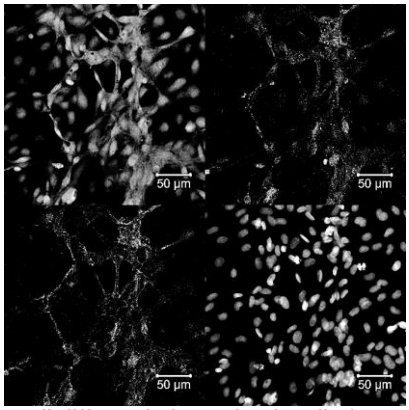

FIG. 2. Cell differentiation using i-Cell A) PECAM-1, B) Selektin E, C) tight junctions, D) cell nuclei

The closer the material is to biological the better. Preliminary results revealed the possibility of effective biofunctionalization with classical polyelectrolytes [3].
For the process of cell differentiation, an additional form of supplement and drug carrier was used in the form of micelles and blood cells. Both of these solutions yielded positive results. At this stage of the research, no significant advantage of the micelle solution or the blood cell solution as a more effective method of delivering the differentiating substance, has been found.

Conclusions

Multifunctional materials are undoubtedly the future of medicine, and polyelectrolytes micelles and erythrocytes are promising materials for effective surface functionalization.

Acknowledgements

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INTERACTIONS OF BIOACTIVE MOLECULES WITH POLIURETHANE SURFACES

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Introduction

Polyurethane is widely used in the medical field due to its unique properties, including flexibility, mechanical strength, abrasion resistance, biocompatibility and customizable chemical properties [1-3]. However, its chemical inertness and hydrophobic nature require surface modifications to improve its biological properties. Functionalization polymeric surfaces by introducing functional groups or altering topography is nowadays a key area of research in polymeric biomaterial [4]. Surface modification using low-temperature plasma is both effective and facile. This approach allows for precise control of the degree of functionalization by adjusting plasma treatment parameters such as gas type (oxidizing, reducing, inert), partial pressure, generator power, and exposure time. Selecting an appropriate plasma gas based on the desired surface properties is crucial.

The use of most commonly used air and oxygen plasma can dramatically improve surface hydrophilicity. NH_3 plasma lead to biocompatibility improvement [5,6]. It should be emphasize that the generated surface functional groups will interact differently with bioactive molecules e.g. drugs. Such interactions are of key importance for drug delivery systems as they influence adsorption/desorption processes, and surface or bulk diffusion, thus controlling drug release kinetics. In this study, the interactions of bioactive molecules with the unmodified and plasma-modified polyurethane surface were investigated using experimental methods and Molecular Dynamics simulations.

Materials and Methods

Medical grade polyurethane films were purchased from American Polyfilm, Inc. The thickness of the polyurethane layer used in the experiments was 100 μm. Before each experiment, polyurethane was washed with 2-propanol (Avantor) and air-dried.

Three different types of biomolecules were selected for the study: ibuprofen, fluorouracil and carbenicillin. These molecules represent different bioactive properties: anti-inflammatory, anti-cancer, and anti-bacterial, respectively, as well as varying functional groups and molecular sizes. The comparison of the structures of the investigated molecules are presented in FIG. 1.

To generate oxygen- or nitrogen- containing functional groups and nanotopography, the polyurethane samples were treated by oxygen and ammonia plasma (FEMTO system, Diener Electronics). The polyurethane samples (before and after modifications) were characterised by spectroscopic methods XPS, SIMS and AFM. MD simulations were carried out using the GROMACS software.

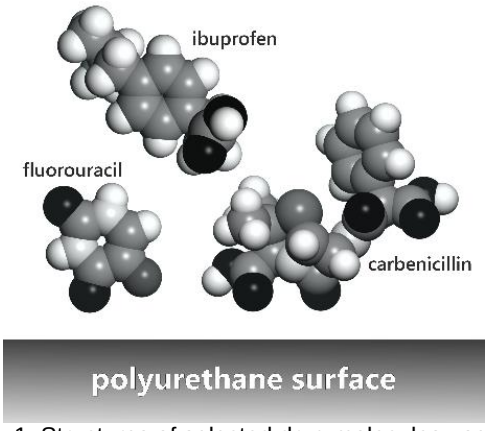

FIG. 1. Structures of selected drug molecules used to investigate their interactions with functionalized polyurethane surfaces.

Results and Discussion

The obtained results clearly demonstrate that while plasma treatment significantly change the surface properties, it does not affect the bulk properties of polyurethane. Spectroscopic methods identified the presence of oxygen-containing surface functional groups (primarily -OH and C=O) and nitrogen groups (-NH_2 and -NH) generated by oxygen and ammonia plasma treatments, respectively. Molecular Dynamics simulations revealed the effect of these surface groups on drug-surface interactions for the selected biomolecules on both unmodified and modified polyurethane surfaces. The results were analysed in terms of the different chemical natures of the surface functional groups and their roles in interfacial processes. MD simulations enabled the interpretation of experimental drug molecule adsorption results at the molecular level.

Conclusions

Plasma modifications effectively functionalize polyurethane surfaces, generating functional groups that influence interactions with biomolecules. The obtained results provide a background for rational designing drug delivery systems that utilise the interactions between bioactive molecules and polymeric substrates, emphasising the critical role of surface functional groups.

Acknowledgements

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OZONATION PROCESS AS AN EFFECTIVE METHOD FOR DESINFECTION AND STERILIZATION OF TEXTILES

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Introduction

Ozone is a form of triatomic oxygen (O_3) with exquisite oxidation properties. Mainly, generated via ozone generators (two main types of ozone generators are corona discharge units and ultraviolet lamps). Either the air or oxygen are used as the feeding gas, however an oxygen generator is required when the air is used as the feeding gas. The ozone gas with very unique oxidation properties has caused research studies on the utilization of ozone for textile applications. Nowadays, ozone is commonly used in various processes such as: denim applications, cotton pretreatments, dyeing and finishing, polyester dyeing and clearing, treatment of various textile fibres (wool, polylactic acid, etc.), and textile wastewater and decolorization process. The work presents the technology of washing in highly ozonated water and also an ozone atmosphere developed by Sovrana Sp.j for the purpose of disinfecting and sterilizing medical textiles. The solution is protected by patent no. P.440194.

Materials and Methods

The research were included the following research cycle:
- preparation and selection the medical textiles samples;
- pre-wash in carbonated, high-ozonated water OWWO (prepared by systems Wofil Robert Muszański), at various times range from 2 to 15 minutes;
- proper washing in OWWO at various times (range 2 to 10 minutes);
- drying in the standard cotton drying cycle.

Then, the microbiological purity of the prepared material samples were tested using the MALDI-TOF technique. Identification of microorganisms was performed using MS MALDI-TOF by the direct transfer method. Determination of the fibres condition after the ozone washing process was examined by the FTIR infrared absorption spectroscopy technique (Thermo Nicolet iS10, measurement using the ATR technique in transmission mode. The microstructure was imaged using SEM (Mira3-FEG-SEM, Tescan).

Results and Discussion

The Quantification of microorganisms on selected textiles samples

Quantitative analysis was performed using the serial dilutions method. In special sterile bags 10g of each tested cotton sample was weighed. The prepared samples were poured thoroughly with 90 ml of 0.9% NaCl mixed. Then, the samples were inoculated onto TSA culture medium in a volume of 100µl. Cultures were performed 10^{-1}, 10^{-2} and 10^{-3}. The plates with the culture medium were incubated for 48 hours at a temperature of 36±1°C.

After incubation, the grown colonies were counted and colonies were selected for identification by MALDI-TOF MS mass spectrometer.

TABLE 1. Quantitative microorganisms analysis.

ID	[cells/1g]	comments
Reference sample	$1 \cdot 10^6$	3different morphological types of bacteria 1mold fungus
Sample washed in OWWO 5 ppm per **5 minutes**	$1 \cdot 10^2$	1type of bacteria
Samples washed in 5ppm OWWO per **10 minutes**	$1{,}9 \cdot 10^2$	1type of bacteria

The conducted research confirmed that the ozone concentration in the OWWO solution used in the washing process is a highly effective process for washing and pre-disinfecting and sterilizing medical textiles. The ozone concentration in water solution equal and above 5 ppm is very effective on spores of fungi and molds, which are often found on medical fabrics and could cause secondary hospital infections resistant to antibiotics.

Conclusions

The research confirms that technology of washing in highly ozonated water and also an ozone purpose of disinfecting and sterilizing medical textiles. Determination of the fibres condition after the ozone washing process confirm that the washing process in safe and effective.

Acknowledgements

Special acknowledgements for Sovrana company for enabling research to be carried out in real industrial conditions.

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BIOMASS FROM *IN VITRO* CULTURES - ITS BIOMEDICAL POTENTIAL

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Introduction

Creating new strategies for the treatment of difficult-to-heal wounds is still a significant challenge. Solutions based on natural components of higher mushrooms, which have the natural ability to absorb and accumulate toxic or stimulating, microstatic, and microbicidal metabolites, such as derivatives of chitosan or antimicrobial peptides, are becoming increasingly popular. Mycelium is also rich in mineral salts and vitamins, which, when properly hydrated, promote cell nutrition. This type of raw material is also a source of proteolytic enzymes that support the digestion of dead tissue. Therefore, there is a strong need to develop controlled mycelium cultures *in vitro* with features that support wound healing. The main goal of this work was to obtain natural material—biomass with health-promoting properties and a specific, repeatable qualitative and quantitative composition—that may be intended for biomedical use. This work aimed to develop repeatable production in laboratory conditions of *in vitro* cultures of three species of mushrooms of the genus *Pleurotus* spp. (*P. citrinopileatus, P. djamor*, *P. ostreatus*) under conditions of controlled absorption of Mg and Zn, supplemented in the form of salts intentionally introduced into the medium at specific concentrations and chemical forms.

Materials and Methods

The research cycle included:
a) modeling and composing the composition of specific liquid media enriched with the addition of selected substances for *in vitro* cultures of mushrooms of the genus *Pleurotus* spp.;
b) conducting controlled in vitro cultures to obtain biomass enriched (fortified) with these substances at the adopted concentration level through the use of Mg and Zn salts.
The biomass for analysis with various sorption parameters was obtained under controlled *in vitro* conditions in a bioreactor on an optimized and modified Oddoux medium. The freeze-dried biomass was then homogenized and subjected to physicochemical tests and microstructural analysis, including SEM and BET tests, necessary to determine the properties of the material—structure, morphology, surface development, size, shape, type of pores, and their number, as well as sorption properties.

The surface area, total pore volume, and average pore diameter of all samples were measured by N2 adsorption using Brunauer-Emmett-Teller (BET) and Langmuir methods (ASAP 2010, Micromeritics, Norcross, USA). The microstructure and surface morphology of mycelium were observed under an SEM microscope (FEI Nova NanoSem 200) with an accelerating voltage of 18kV and magnification of 10,000 for in vitro culture.

Results and Discussion

The BET specific area of the examined mycelium was 1.9 m²/g for *P. djamor* (**Pdj**), 1.5 m²/g for *P. citrinopileatus* (**Pc**), and 13.5 m²/g for *P. ostreatus* (**Po**). The surface areas obtained from the Langmuir equation were 2.2 m²/g for Pdj, 2.8 m²/g for Pc, and 20.2 m²/g for Po. Total pore volume (V_{micro}), micropore area (S_{micro}), and average pore diameter of mycelium are listed in Table 1.

TABLE 1. Analyzed parameters.

Type of in-vitro culture / parameters	Pdj	Pc	Po
Vmicro, cm^3/g	0.0003	0.0002	0.0025
Smicro, m^2/g	0.4	0.2	4.2
Average pore diameter, nm	14	6	12

The research confirmed that the microstructure of the material significantly impacts the accumulation process of the bioelements with health-promoting properties. Their degree of accumulation in the mushroom mycelium increases with the development of the surface.

Conclusions

The research confirms that mushrooms are not only functional food with documented health-promoting properties but also an interesting biological material. The obtained results constitute an important contribution to the development of biomaterials engineering sciences. Biomass from in vitro cultures can be a competitive raw material with unique physicochemical properties enriched with selected bioelements, including Mg and Zn, used as a material for personalized dressings.

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FORMATION AND CHARACTERIZATION OF CALCIUM PHOSPHATE CEMENTS MODIFIED WITH MAGNESIUM IONS FOR BONE REGENERATION APPLICATIONS

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Introduction

Calcium Phosphate Bone Cements (CPCs) play a crucial role in advanced regenerative medicine, particularly in orthopedics and dentistry. Since their introduction in the 1980s, CPCs have gained recognition as biomaterials capable of effective bone repair and reconstruction due to their bioactivity, biocompatibility and ability to stimulate osteointegration [1]. Bone cements are biomaterials created by mixing a powder phase with a liquid phase, forming a moldable mass that can be implanted into the body. In the context of joint replacement and attaching prostheses to bone, bone cements serve as fillers that secure the implant to the bone [2]. Crucially, the tight connection between the cement and the bone, as well as between the cement and the prosthesis, facilitates the optimal distribution of compressive forces and strain energy at the interface [3]. The primary role of bone cements is to efficiently transfer loads between the implant and the bone, ensuring the durability and functionality of the implant [4].

Materials and Methods

The aim of the presented research is the formation of cements based on calcium phosphates with Mg^{2+} ions. The cements were formed from synthesized ceramic powders with Ca/P ratios of 1.0, 1.5 and 1.67, respectively. The ceramic powders were synthesized by the wet precipitation method from calcium nitrate and disodium hydrogen phosphate salts and the modifier magnesium sulfate. Cements were formed by mixing the ceramic powder with phosphoric acid and citric acid for 15 seconds. The formed cements were then subjected to Fourier transform infrared spectroscopy (FT-IR) studies to determine the functional groups. In addition, the crystallinity of the resulting cements was checked using X-ray diffraction (XRD) and the surface morphology was examined using Scanning Electron Microscopy with energy-dispersive X-ray spectrometry (SEM/EDS).

Results and Discussion

Bone cements formed from ceramic powder with a calcium/phosphorus ratio of 1.5 had the best formulation due to their applicability. The FT-IR spectra of the cements studied indicated elevated intensities due to magnesium ions. In XRD studies, the influence of magnesium ions on the crystallinity of the tested cements is evident. In SEM images, differences in grain size depending on the Ca/P molar ratio are noticeable.

Conclusions

It can be concluded that using the described method it was possible to obtain bone cements with Mg^{2+} ions. Calcium phosphate-based bone cements are an integral part of modern bone regeneration strategies. Their development and modification represent a promising research direction to improve the quality of life of patients with bone damage. Further research into optimizing composition and synthesis conditions has the potential to lead to the development of more advanced materials for bone regeneration applications.

Acknowledgements

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OPTIMIZATION OF SILVER NANOPARTICLES PREPARATION IN-BATCH AND ON-CHIP BY BIOREDUCTION AND STABILIZATION WITH *VERBENA* EXTRACT

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Introduction

The green synthesized nanoparticles of metals which are monodispersed, having specific sizes and shapes have gained importance in biomaterial science. The secondary as well as primary metabolites of plants are continuously involved in reduction process to produce environmentally friendly nanoparticles. Extracts of plants contain alkaloids, phenolics, flavonoids, and terpenoids, that are primarily accountable for reduction of metal ions into metal nanoparticles, and the stabilization in one step [1,2]. The synthesis of metal nanoparticles takes place using the traditional in-batch method allowing the production of large quantities of nanoparticles at a time, but controlling the reaction conditions in large volumes can be difficult, which can lead to non-uniformity in the size and shape of the nanoparticles. On-chip synthesis, on the other hand, usually takes place in microreactors, which allows more precise control of the reaction conditions, which can lead to better stabilized and smaller nanoparticles with quantitative yield of reduction [3, 4]. The aim of this study was, to evaluate the size and stability of Ag nanoparticles synthesized using verbena extract, prepared in-batch and on-chip.

Materials and Methods

Lemon verbena leaf (*Aloysia citrodora Palau folium*), and verbena top plant (*Verbena officinalis L.*) were processed to obtain simple water extracts and then mixed with $AgNO_3$ (CAS 7761-88-8; Mach Chemikalie). The plant leaf (L) and top plant (TP) aqueous extract solutions were prepared by weighting 2.5 g of plant leaves, top plant of both separately in 100 mL of distilled water (T=100 °C) for 10 min followed by filtration using Whatman no. 1 filter paper). Filtrate solutions were then mixed with 5, 15, 30, and 60 mM silver precursor in a volumetric ratio 1:1 and left under continuous stirring (250rpm) for t=30. The 3D printed microreactors (FIG. 1) from PLA were used for on-chip silver nanoparticles bioreduction at flow rates 20 ml/h.

FIG. 1. 3D-printed microreactor.

The primary detection of AgNPs was carried out by visual observations of colour change after treatment of verbena extract with $AgNO_3$. In order to, assess the stability of the colloidal nanoparticles, a zeta-potential test was carried out. The morphology and size uniformity were studied using scanning transmission electron microscope (STEM) to evaluate and conclude the effectiveness of various process parameters on the properties of silver nanoparticles. Additionally, particle measurements using imageJ software were carried out.

Results and Discussion

Experiments in a batch arrangement point to a better bioreduction function when using a top plant than when using a leaf only, as confirmed by the zeta potential results. When using a leaf, significantly larger particles (30-90 nm) are produced (FIG. 2a), on the contrary, when using a top plant, smaller nanoparticles (6-23 nm) and better stabilized are formed. The leaves probably contain more phytochemicals responsible for the rapid reduction of nanocrystals, while the top plant has a higher concentration of stabilizing components. According to monitoring the morphology of the nanoparticles by the STEM method, it is clear that the best result on the chip was achieved at the smallest concentration of silver precursor 5mM (18-74 nm) (FIG. 2b). On-chip mixing seems to promote quantitative reaction progress, so we can afford to lower the precursor concentration and get a better yield, as in the case of nanoparticle reduction in a batch setup. On the other hand, at higher concentrations of precursors, two size fractions appear, elongated interconnected shapes, and finally at 60 mM a lot of dendritic clusters. Although post-reactions still take place in the collection vessel after passing through the chip channels, mixing on the chip has a major effect on the size and stability of the nanoparticles.

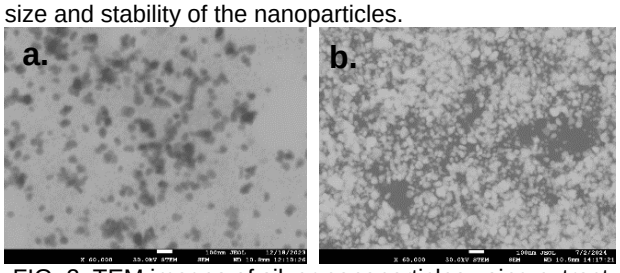

FIG. 2. TEM images of silver nanoparticles using extract of *Aloysia citrodora Palau folium* prepared: a. in-batch, b. on-chip

Conclusions

The synthesis of nanoparticles using the top plant combined with on-chip technology is more efficient and leads to more stable and smaller nanoparticles compared to traditional in-batch synthesis using leaves.

Acknowledgements

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LIMITING THE CYTOTOXIC EFFECT AND RELEASE OF METAL IONS FROM SLM-PRINTED HIGH-POROUS Ti6Al4V IMPLANTS BY DIP-COATING WITH CHITOSAN + BERBERINE

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Introduction

Highly porous implants manufactured using the additive method, most often from Ti6Al4V, are increasingly used in clinical practice. Replacing a permanent implant with a scaffold allows for a stable connection between the implant and the bone, allowing tissue to grow inside the implant [1]. Despite the increased functionality, surface modifications of highly porous implants are a confession. Ti6Al4V is characterized by very good biocompatibility and high corrosion resistance. Nevertheless, to ensure the best possible cooperation with the body, the use of additional barrier coatings in the form of biodegradable polymer coatings can bring many benefits by temporarily limiting the contact of metal with tissue and providing substances that are beneficial from the point of view of cells and tissues [2].

Materials and Methods

The research was carried out on samples in the form of highly porous SLM-printed Ti6Al4V implants, subjected to PEO modification aimed at surface functionalization by creating a passive layer with a specific topography and chemical composition [3]. The samples were dip-coated by incubating for 24 hours in a 1.5% chitosan solution suspended in 2% acetic acid with the addition of an active substance. The tests were carried out on samples coated with chitosan (Cht) alone and chitosan with addition of berberine in high (25%) and low (0.3%) concentrations (BBR high, BBR low). The coated samples were dried at room temperature with forced air circulation. The samples were sterilized with a high-energy electron beam (25 kGy).

The ion release study was performed using ICP-AES spectroscopy. Measurements were carried out on PBS solutions in which the samples were incubated for 2, 4 and 6 weeks at a temperature of 37 degrees.

Cytotoxicity analysis was performed in accordance with the ISO 10993-5 standard. Mouse fibroblasts (L-929; ATCC) were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and 10 mM HEPES pH 7. Cells were incubated at 37°C, 5% CO2. The samples were incubated in EMEM medium (3 mL/cm^2) at 37 °C for 24 h. After this time, dilutions of the extracts were prepared (2x, 4x, 8x, 16x and 32x) and added to the cell cultures. Cell suspension (100 µL) with a density of 4 × 104 cells/mL was seeded onto a 96-well plate and incubated for 24 h. Then, the medium was removed and the medium containing the extract of the tested samples was added. Cell viability was assessed after 72 h incubation using the Cell Counting Kit – 8 (CCK-8) and In Vitro Toxicology Assay Kit, Sulforhodamine B based (SRB) tests.

Results and Discussion

The results of spectrophotometric measurements showed a significant impact of the applied surface modifications on the amount of metal ions released into the environment. For all samples covered with a polymer layer, no metal ions were detected after 2 weeks of implant incubation. Although the number of determined ions increased with the incubation period, their number was lower than for unmodified implants and those subjected only to electrochemical modification. The best results were obtained for implants covered only with chitosan. The addition of berberine decreased the barrier effect of the coating as the concentration increased.

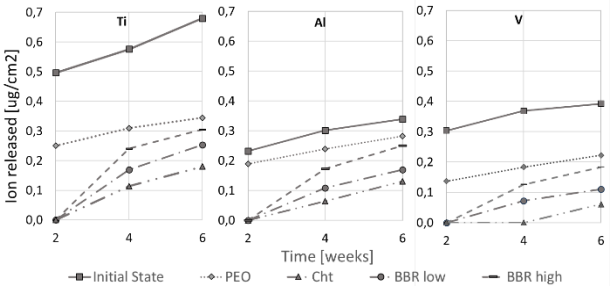

FIG. 1. Graph of the ion density released into the incubation solution over time for Initial States, PEO, Cht, BBR high and BBR low samples.

Studies have shown that implants after PEO have cytotoxic effects. 2x dilution of the PEO extracts allowed for the cell survival >70%. The concentration of berberine in BBR high samples was cytotoxic. Cellular tests showed a positive effect of the applied chitosan and chitosan with low concentration of on cell survival, which was >100% compared to K$^-$.

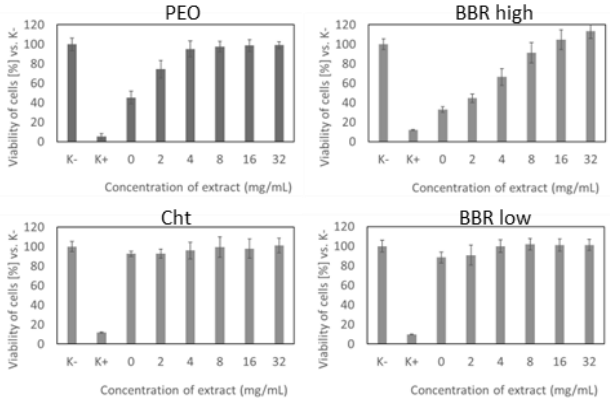

FIG. 2. The effect of the extract obtained from implants after PEO and covered with a polymer layer (Cht, BBR high, BBR low) on the proliferation of mouse fibroblasts compared to the control group (cells cultured in standard conditions).

Conclusions

The use of chitosan and chitosan+bernberine coatings significantly reduced the amount of ions penetrating from the tested implant into the environment imitating the in vitro environment. Berberine in high concentrations has a cytotoxic effect on fibroblast cells. Chitosan coating and chitosans with the addition of a low concentration of berine significantly improve the survival and proliferation of fibroblast cells.

Acknowledgements

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BIOACTIVE GLASS NANOPARTICLES IN TERNARY OXIDE SYSTEM (SiO_2-CaO-P_2O_5)

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Introduction

Bioactive glasses (BG) are highly reactive materials, that can bond with living tissues, generating a specific biological response. They have controlled degradability and the capability to stimulate new bone formation. What is unique in bioactive glass nanoparticles (BGNs) is their small size, large specific surface area and large surface-to-volume ratio. They can be used in biomedical applications such as in drug delivery. Furthermore, BGNs can be used as modifiers of polymer composites, improving their physicochemical and biological properties. However, developing BGNs is challenging, especially when it comes to their morphology and oxide composition. The selection of appropriate precursors and manufacturing techniques allows for controlling these characteristics.

Materials and Methods

In this study bioactive glass nanoparticles were synthesized and characterized. The glasses come from ternary oxide system and differ significantly in SiO_2:CaO molar ratio: A2 (40% mol SiO_2, 54% mol CaO, 6% mol P_2O_5) S2 (80% mol SiO_2, 16% mol CaO, 4% mol P_2O_5). The primary objective was to optimize the sol-gel method to obtain bioactive glass nanoparticles with assumed compositions. SEM/EDX was used to assess morphology and composition of the BGNs. Structural analysis (FTIR, XRD) was conducted to confirm glassy character of the materials. An apatite-forming ability of the BGNs upon incubation in simulated body fluid was examined using SEM/EDX, FTIR, ICP-OES methods.

Results and Discussion

The nanoparticles obtained have uniform shape and size. The assumed composition of BGNs was obtained by manipulating the synthesis parameters. This also resulted in different particle size and agglomeration tendency. Samples with high SiO_2 content (S2) were amorphous, while A2 materials demonstrated a glass-crystalline character. SBF test demonstrated high bioactive properties in both S2 and A2 glass nanoparticles just after 3 days of incubation.

Conclusions

Bioactive glass nanoparticles are promising materials, but they require further investigation in a field of glass composition and agglomeration of nanoparticles.

Acknowledgements

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EFFECT OF LIQUID RUBBER MODIFICATION ON STRESS DISTRIBUTION ON DENTAL COMPOSITE FILLINGS: A FINITE ELEMENT ANALYSIS

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Introduction

Dental composites have become the preferred choice for filling, and restoring lost tooth tissue, accounting for over 70% of all such procedures. This is due to several factors, including their ease of application, rapid curing, and excellent functional and aesthetic properties. Nevertheless, dental composites do not provide a permanent replacement for restored tooth tissue, with an average clinical lifespan of approximately five years [1]. The limited durability of these materials is primarily due to their susceptibility to fracture under occlusal forces. One promising direction for improving fracture toughness is to modify the matrix with liquid rubber. An important issue is also to determine the stress distribution in the filling area depending on the type of material used.

Materials and Methods

Finite element simulation was performed using Abaqus software (Simulia) on the tooth model created in the CAD software. A two-layer filling was employed, comprising a flow-type filling in the dentin area and a classic composite in the enamel one. The total load of 700 N was applied in a manner consistent with the distribution of occlusal forces observed in a molar, as evaluated using a T-scan Novum device (Tekskan, USA) (FIG. 1). Two simulations were conducted. The first involved a filling using reference (unmodified) composites, while the second considered composites modified with liquid rubber. The simulation employed material data for two experimental dental composites, namely flow and classic types, which contain in their matrix a mixture of resins with the following composition: 20% w/w BisGMA, 30% w/w BisEMA, 30% w/w UDMA, and 20% w/w TEGDMA. The reinforcement consisted of Al-Ba-B-Si glass, Ba-Al-B-F-Si glass with particle sizes of 0.7 and 2 μm, and pyrogenic silica (20 nm). The reinforcement content was 50% by volume in the flow composite and 80% in the classic one. Furthermore, all composites were formulated with a photoinitiation system (CQ: DMAEMA). Liquid rubber Hypro 2000X168LC VTB (Huntsman International LLC, USA) was incorporated as a modifier at a concentration of 5% by weight relative to the matrix. The simulation yielded the distribution of principal stresses.

Results and Discussion

For the reference filling (FIG. 2a), principal tensile stresses of approximately 25 MPa were observed at the interface between the filling and the enamel, while the highest compressive stresses occurred at the top of the nodule and had a value of 53 MPa. In the case of the filling modified with liquid rubber (FIG. 2b), the highest tensile stresses, around 20 MPa, were observed at the enamel surface adjacent to the filling. The analysis indicated that modifying the composites with liquid rubber allows for a reduction in tensile stresses by about 5 MPa, especially in areas exposed to direct occlusal forces (FIG. 2a). The modification of the filling material resulted in the accumulation of tensile stresses primarily in the enamel areas rather than in the filling itself. Conversely, for the reference filling, a significant portion of the compressive stresses, amounting to 8 MPa, was transferred to the filling and the filling-enamel interface. This is an unfavorable phenomenon as it leads to increased material strain and, consequently, earlier cracking and degradation of the filling, negatively impacting its durability. Filling modified with liquid rubber enables a more favorable distribution of compressive stresses within the filling (reduced by 50%), thereby lowering the risk of cracks and mechanical failures. These findings suggest that modifying dental composites with liquid rubber could be an effective strategy to enhance their strength and longevity, which is crucial for dental practice.

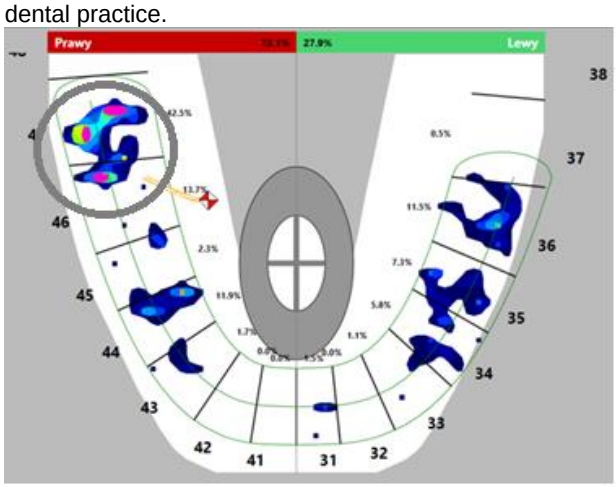

FIG. 1. Distribution of bite forces determined using a T-scan device.

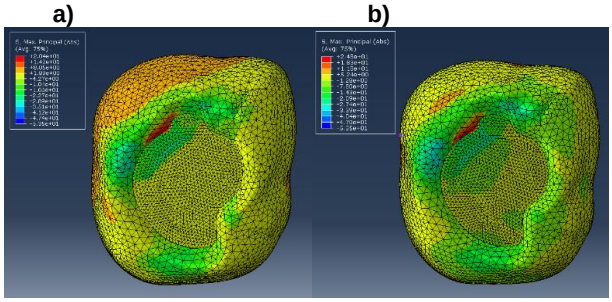

FIG. 2. Stress distribution around the tooth filling: a) composite without modification b) composite modified with the addition of 5% liquid rubber.

Conclusions

Modification of dental composites with liquid rubber results in about 50% stress reduction in the filling area and more favorable stress distribution.

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EXTRACTION OF THE EXTRACELLULAR MATRIX WITHOUT THE USE OF DETERGENT. ANALYZING THE DIFFERENCES BETWEEN CHEMICAL AND PHYSICAL METHODS FOR OBTAINING DECELLULARIZED EXTRACELLULAR MATRIX (dECM)

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Introduction

The extracellular matrix (ECM) is a complex network of proteins and polysaccharides that provides scaffold and a supportive environment for cells. In regenerative medicine, decellularized extracellular matrix (dECM) is derived from native tissues and preferred over chemically synthesized biomaterials. Researchers are increasingly using dECM as a bioink component in 3D bioprinting due to its excellent biocompatibility and biodegradability. Preserving key dECM components such as collagen, glycosaminoglycans (GAGs) and growth factors while reducing immunogenicity is the primary challenge in decellularization techniques. The analysis of previously used tissue decellularization methods allowed us to discover that the type of method directly affects the residual components of dECM. The existing types of decellularization describe biological, chemical and physical methods. The aim of the study was to demonstrate that effective decellularization of soft organs is possible without the use of toxic detergents.

Materials and Methods

The biological material consisted of pig pancreases. The material was ground and used for different types of decellularization. These were experiments using chemical methods (Triton X-100, sodium deoxycholate (SDC), PBSx1 solution, isopropanol solutions), biological method (lipase digestion) and physical methods (homogenization-centrifugation (HC), freezing and thawing (FTH), Supercritical Extraction CO_2 ($scCO_2$). Prior to $scCO_2$, the material was freeze-dried. In cases where the material was rinsed in solutions, freeze-drying was one of the final stages of the experiment. Then the material was ground using a cryogenic grinder. For each sample, the content of: dsDNA, fat (Soxhlet method), collagen and GAGs was determined.

Results

Compared to the native pancreas with a dsDNA concentration of 638.53 ± 45.17 ng/mg, decellularization by the $scCO_2$ resulted in a decrease of dsDNA content to 332.06 ± 6.34 ng/mg. The dsDNA concentration in dECM obtained through other decellularization methods was significantly below the upper limit of generally accepted 50 ng/mg standard [1]. The fat content in the native pancreas was 17.96 ± 0.96% and decreased to < 4% using Triton X-100 solution. Using individual methods fat was 4.38 ± 0.16% for HC, 8.65 ± 0.88% for rinsing in SDC, and 13.06 ± 0.84% for $scCO_2$. After using other decellularization methods, no reduced fat was observed. The collagen concentration was as follows: for dECM after rinsing in Triton X-100 was 901.46 ± 69,34 µg/mg, PBSx1 rinsing: 561.58 ± 12.17 µg/mg, lipase digestion: 505.12 ± 2.56 µg/mg, rinsing in isopropanol: 458.42 ± 41.90 µg/mg, SDC rinsing: 384.59 ± 31.67 µg/mg, FTH: 184.00 ± 8.25 µg/mg. The other methods resulted in lower collagen content but were still satisfactory. Respectively, for HC was 70.38 ± 0.44 µg/mg, $scCO_2$ was 51.64 ± 0.55 µg/mg. Determination of GAGs content indicates the highest level of this component in the pancreas decellularized by $scCO_2$: 8.74 ± 0.05 µg/mg and HC: 4.90 ± 0.01 µg/mg, other methods resulted in a very low amount of GAGs (< 1 µg/mg).

Conclusions

The most optimal decellularization method was the detergent-free method using homogenization-centrifugation (HC). The dECM obtained in this way was characterized by a low content of dsDNA and fat, and a relatively large amount of GAGs. These features and the lack of the use of chemicals make it the most promising dECM variant as a component of bioinks for 3D printing. And it can be used in regenerative medicine in the future. However, depending on the type of method used, there are differences in the composition of the obtained extracellular matrix. Chemical and biological rinsing methods resulted in low dsDNA content and high collagen abundance, which indicated effective removal of cellular elements from the research material. Although in this case, the low level of GAGs may indicate the elution of lower molecular weight dECM components. This reduces its biological properties.

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DEGRADATION OF ANODIC OXIDIZED TITANIUM ALLOYS UNDER CONDITIONS OF ELECTROSTIMULATION OF BONE UNION

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Introduction

One method of promoting bone union, in cases of its disorder, is electrostimulation. It involves replacing the naturally generated functional currents in the bone as a result of loading it, with currents generated by an electrostimulator. Bone union disorders occur in cases of stabilization of bone fragments with the use of metal implants. The current flowing, in addition to tissue and body fluids and mechanical factors, can be a contributing factor to the corrosion processes of implants stabilizing bone fragments.

Therefore, the purpose of this study was to determine the effect of bone union electrostimulation conditions on the degradation process of anodically oxidized titanium alloys, a material commonly used for implants in orthopedics and traumatology.

Materials and Methods

The simulated in vitro bone union electrostimulation procedure was conducted in Ringer's solution at T = 37°C, for 28 days. The tests were conducted for both undeformed and previously deformed specimens in the elastic range in a three-point bending test.

Applied were:

(i) sinusoidal current with amplitude A = 8 mA and frequency f = 60 kHz,

(ii) a sequence of alternating pulses A exp-(t/t0) with amplitude A = 80 μA and time constant t0 = 0.2 and frequency f = 1 Hz.

Sinusoidal current (i) electrostimulation corresponded to a non-invasive, capacitive method of electrostimulation of bone fusion. The alternating pulses (ii) correspond to the currents generated in long bones during walking, while the electrode configuration of the non-invasive electrostimulation method.

The scope of the study included observations of surface topography using light microscopy and optical profilometry, potentiodynamic corrosion resistance tests and the concentration of metal ions passing into Ringer's solution with 28 days of electrostimulation. The tests were carried out for samples exposed only to Ringer solution (without current) and those exposed to electrostimulation conditions.

Results and Discussion

The conditions of electrostimulation of bone union affect the kinetics of the degradation processes of the surface layers formed on the substrate of the two titanium alloys studied. There is an increase in the concentration of elements penetrating the Ringer's solution with a concomitant increase in the corrosion resistance of the samples (corrosion potential, polarization resistance) compared to samples exposed only to the solution (without current flow). Observations of the surfaces of the specimens showed no significant effect of bone fusion electrostimulation conditions on their surface topography. This is true for sinusoidal and pulsed currents of both undeformed and deformed specimens regardless of how the surface layer was produced.

Conclusions

The course of degradation of the surface layers produced on the substrate of Ti6Al4V and Ti6Al7Nb alloys under conditions of electrostimulation of bone union indicates that the studied surface layers provide good protection of implants against pitting corrosion. This applies to undeformed surface layers as to deformed ones regardless of the applied current.

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MECHANICAL PROPERTIES OF METAMATERIALS USED FOR ATRAUMATIC TOOLS

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Introduction

In order to avoid tissue damage during surgeries, only minimal operational forces should be used on tissues during grasping and clamping. Currently, only geometrical regulators for excessive forces are used (stop pins, atraumatic inserts etc., special atraumatic serration and shape of the jaws). The key issue will be achieved by metamaterials with increased reversible, spring-like deformation potential, whereby the deformation is in detail controllable by the used lattice structure and even enables a fully reversible cyclic deformation with “ideal-elastic – ideal-plastic” behaviour for metals (by chiral and anti-chiral materials). For such an application of metamaterials, shown potentials are i.e. (i) shape-matching features for design soft grippers for touching delicate objects with the maximum surface contact and, thus, minimum contact force [1], (ii) shape-shifting features, (iii) biomimetic mechanical metamaterial and biomimetic multifunctional structures, etc. [2]. There is no data available about the application of metamaterial for atraumatic effect.

Consequently, the strategy here is to focus on so-called “chiral” and “anti-chiral” mechanical metamaterials, which, upon deformation, show an intrinsic overload protection in their stress-strain diagrams [3-5]. Hence, their use as e.g. 'counter-springs' in the working parts of clamps and laparoscopic instruments described above, reduces their compressive force and potentially avoids traumatic clamping and grasping. Although the general concept of chiral and anti-chiral metamaterials is present in literature, most of these studies were conducted on polymer structures with dimensions in the order of several centimeters. The challenge and main innovation in this researcht is to apply this feature to metallic structures with significantly smaller dimensions. The change from polymer to metal changes the behavior of the parent material significantly (viscoelastic vs. elastic-plastic material behavior).

Materials and Methods

Thus, the study attempted to assess the mechanical properties of the metamaterials with different structure made by Ti alloys. Static and dynamic testing tests were performed as part of the mechanical property use MTS system and Alemnis. Additionally, the wear resistance was measured using the ball on disc method. Measurements of nano-hardness and Young modulus were made by means of Oliver & Pharr method with application of Berkovich intending tool (Triangular base pyramid). The surface morphology of the prepared samples was evaluated with the use of an Atomic Force Microscope (non-contact mode). The scanned area was 10 × 10 μ mat the resolution of 256 × 256. Additionally, observation was carried out with the use of scanning electron microscope TESCAN with secondary electron (SE) detection, within the magnification range of 1000–100,000x. Furthermore, surface roughness tests were performed with a Leica DCM 8 optical profilometer

Results and Discussion

The obtained data showed different mechanical properties depended of structure of metamaterials FIG. 1 and 2. The knowledge gained from this research has practical importance for application of metamaterials on atraumatic tools.

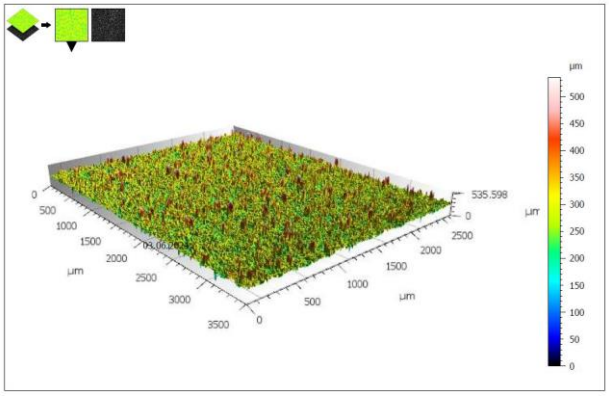

FIG. 1. Example of metamaterial sample surface topography.

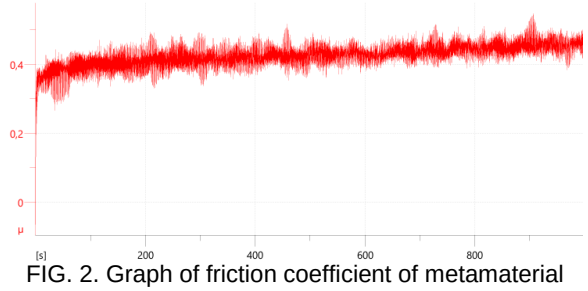

FIG. 2. Graph of friction coefficient of metamaterial Ti alloy sample.

Acknowledgements

The project was financed by the Polish National Center for Research and Development granted under the M-ERA.NET3/2022/75/AtraumaBioMat/2023 contract as part of the M-ERA.NET 3 Call 2022 competition; Austrian Federal Ministry – FFG grant number: FO999900377; Scientific and Technological Research Council of Türkiye: Tübitak Ardeb 1071 Programme grant number:123N419

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BIOACTIVE BORATE GLASSES AS THERAPEUTIC ION (Zn^{2+}) CARRIERS

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Introduction
Borate bioactive glasses (BBGs) may undergo faster conversion to hydroxycarbonate apatite (HCA) both in vitro and in vivo compared to silica-based glasses due to their higher solubility. BBGs promote cell proliferation and differentiation in vitro, as well as tissue infiltration in vivo, by degrading at a rate that is tailored to the rate of tissue regeneration. Depending on their composition, borate glasses can also be modified to achieve controlled and linear therapeutic ion release profiles, such as zinc, which plays a role in bone metabolism. Their ability to rapidly release ions also allows for their use in wound healing.

Materials and Methods
The study aims to evaluate the effect of the synthesis method and the presence of the modifier (zinc) on glass microstructure, morphology, and bioactive properties in vitro. Zinc-doped BBGs were obtained using traditional melting and the sol-gel method with various organic boron precursors (triethyl borate – TEB, tributhyl borate – TBB, trimethyl borate – TMB, trimethoxyboroxine – TMBx). The glass composition was $40B_2O_3$-(54-x)CaO-$6P_2O_5$-xZnO mol%, where x was 0 and 5. The phase composition and crystallization tendency were assessed using X-ray diffraction (XRD), while structural characteristics were provided through infrared spectroscopy (FTIR). A simulated body fluid (SBF) assay was performed to determine the in vitro bioactivity of the obtained materials. Scanning electron microscopy (SEM) with EDS microanalysis was performed to analyze the morphology and chemical composition of the samples before and after incubation. Ion release kinetics (B, Zn) and changes in the concentrations of Ca and P ions in the incubation solution were investigated using inductively coupled plasma atomic emission spectrometry (ICP-OES).

Results and Discussion
XRD analysis proved the amorphous nature of glasses obtained by the sol-gel method, while melt-derived samples exhibited crystallization of calcium phosphates. FTIR spectra showed the presence of boron in the obtained glasses in both tetrahedral and trigonal coordination. Neither the glass synthesis technique nor the type of boron precursor was observed to have a significant effect on the structural characteristics. Both FTIR spectra and SEM-EDS analysis showed the formation of an apatite layer after incubation in SBF, confirming the in vitro bioactivity of the materials. ICP-OES also confirmed the bioactive properties of the glasses. The materials with ZnO showed the lowest tendency to form calcium phosphates.

Conclusions
The results indicate high application potential for the obtained materials in the field of tissue engineering.

Acknowledgements
This work was supported by the program „Excellence initiative – research university" for the AGH University of Krakow.

CARBONATE ERYTHRITOL FOR THE SYNTHESIS OF NON-ISOCYANATE POLYURETHANES AS NEW BIOMATERIALS

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Introduction

Polyurethanes are the most versatile and common polymer materials. In the synthesis of conventional polyurethanes, ether or ester polyols, diisocyanates and a low molecular weight chain extender are used. The synthesis of these substrates, their subsequent polymerization and recycling of used polyurethanes undoubtedly have a toxic effect on the environment. The synthesis of diisocyanate, which is obtained from deadly and energy-intensive phosgene, is a toxic process. Moreover, the susceptibility of diisocyanate to hydrolysis adversely affects the environment and human life. Due to the growing ecological awareness, the search for an alternative method of PU synthesis began, aiming at elimination of the use of isocyanates [1]. A balanced approach is the synthesis of isocyanate-free polyurethanes, which are obtained from cyclic carbonates and diamines. Cyclic carbonates can be synthesized with 1,2-diols or can be formed by introducing CO_2 into epoxy compounds, which can be also synthesized from renewable raw materials [2].

Materials and Methods

(2R,3S)-butano-1,2,3,4-tetraol (erythritol) was obtained from Wish Pharma. Dimeric fatty acid (P1075) was obtained from Croda. Dimethyl carbonate, 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), hexamethylene-diamine (HMDA) and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich.

Results and Discussion

Synthesis of erythritol bis(carbonate)

In a 100 ml flask, erythritol (2 g, 16.4 mmol) and TBD (114 mg, 0.820 mmol) were dispersed in DMC and heated to 60°C for 40 min until complete dissolution at the rotary evaporator. Then the temperature was lowered to 40°C and the reaction continued at 314 mbar. After 30 min, a white precipitate erythritol bis(carbonate) was formed. The product was filtered off after the mixture was cooled down to room temperature and washed with water yielding a white powder (1.5 g, 52,6%). FIG. 1 shows the course of EDC synthesis.

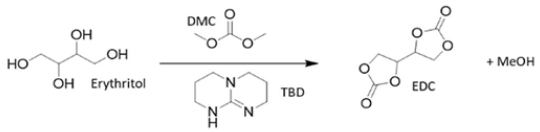

FIG. 1. Synthesis of EDC by transesterification of erythritol with DMC using TBD as an organocatalyst.

NIPU's were prepared by carrying out the bulk and solvent reaction.

Synthesis carried out in a solvent ($NIPU_{solvent}$)

2 g of erythritol carbonate and 5 ml of DMSO were placed in a three-necked flask on a magnetic stirrer with heating function. Dissolution of erythritol carbonate in DMSO was carried out at 80°C. Inert gas was also supplied to the system. After the erythritol carbonate was completely dissolved, the appropriate amount of P1075 was added and stirring and heating continued. In order to control the progress of the reaction, FT-IR spectra of the samples were taken. When the reaction stopped, hexamethylenediamine was added. The P1075/HMDA molar ratio was 9:1. After mixing, the reaction mixture was poured into a mold and placed in a vacuum oven set at 80°C for three days.

FT-IR was used to control the progress of the reaction and to confirm the structure of the obtained NIPU materials. The band around 3300 cm^{-1} comes from stretching vibrations of -OH groups and stretching vibrations of secondary amine groups characteristic of urethanes. The decrease in the intensity of the band at 1800 cm^{-1} originating from the stretching vibrations of carbonyl bonds in the erythritol carbonate ring is observed, in favor of an increase in the intensity of the band of stretching vibrations of carbonyl bonds in urethane groups at 1705 cm^{-1}. The obtained FT-IR spectra of the final elastomers show the complete disappearance of the band at 1800 cm^{-1} originating from the stretching vibration of the carbonyl group in the cyclic erythritol carbonate. This proves the correct course of the reaction and obtaining the isocyanate-free polyurethane NIPU. Both carried out reactions of the formation of NIPU proceeded similarly, however, the reaction carried out in the bulk lasted much shorter. Thermal degradation of NIPUs was investigated using TGA under nitrogen atmosphere. All the prepared NIPUs were thermally stable up to ca. 200°C with the initial decomposition temperature (T_p) of 210 and 208°C for $NIPU_{(solvent)}$ and $NIPU_{(bulk)}$, respectively. The thermal degradation of obtained NIPUs was shown to proceed in three main steps, typical for this type of polyurethane materials. The first weight loss takes place at 289 and 225°C for $NIPU_{(solvent)}$ and $NIPU_{(bulk)}$, respectively, and it corresponds to the degradation of the urethane linkages. The second (388 and 390°C for $NIPU_{(solvent)}$ and $NIPU_{(bulk)}$, respectively) and third (461 and 454°C for $NIPU_{(solvent)}$ and $NIPU_{(bulk)}$, respectively) weight loss is probably attributed to the decomposition of polyether amine chain and ether bonds cleavage in the structure of cyclic carbonate.

Differential scanning calorimetry was used to determine the glass transition temperature (T_g) of the prepared materials and checking whether the obtained materials exhibit thermoplasticity. The glass transition temperatures for $NIPU_{(solvent)}$ and $NIPU_{(bulk)}$ are -26.4°C and -15.6°C, respectively, and differ by almost 11°C. Both materials showed a glass transition temperature below zero, which proves the elastomeric properties of the obtained NIPU materials.

Conclusions

Erythritol di(carbonate) was obtained from *meso*-erythritol featuring mild reaction conditions and a facile workup in 53% yield. The structure of the obtained EDC was confirmed by FT-IR, ^{1}HNMR and ^{13}CNMR analysis. The preliminary reactions of using the obtained EDC to synthesis the NIPU showed its highly reactivity enabling the solvent-free and not catalytic reaction. FTIR study confirmed the reaction between cyclic carbonates and amines. The materials showed high thermal stability up to 208°C.

Acknowledgements

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ADVANCED THERAPY MEDICINAL PRODUCT (ATMP) – THE REGULATORY PATH FROM CLINICAL TRIAL READINESS TO MARKET APPROVAL

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Introduction

In recent years, there has been a dynamic development of scientific research in biotechnology, bioengineering and biomedicine. In Poland, the impetus for the development of research in the aforementioned fields was the establishment of funding institutions. On the other hand, a second significant factor influencing the development of clinical research in Poland was the establishment of the Medical Research Agency in 2019. The mission of the Agency is to create an innovative healthcare system (by funding research) and to build awareness about clinical trials. Thanks to the above initiatives, many academic institutions and research centers have undertaken research in medical biotechnology, as well as regenerative medicine. These fields seek to create personalized medical products or therapies to replace damaged tissues with functional ones using tissue engineering and molecular biology methods. A key element in the implementation of such therapies is the conduct of a clinical trial. By 2019, there were about 18 clinical trials of advanced therapy medicinal products in Poland. Most of these trials were non-commercial [1], [2]. A clinical trial collects clinical data on safety and preliminary efficacy, on the basis of which regulators can decide whether to approve a therapy for market use. Marketing approval is granted in Europe by the European Comission baedon EMA opinion. The aim of this review is to provide overview of market authorisation procedure of ATMPs' in Europe.

Materials and Methods

A literature review of ATMPs and their characteristics was conducted in the PubMed database for matched English-language articles. The search was performed using keywords, combining the words: "ATMP", "advanced therapy medicinal product", "CGT", "RMAT", "regenerative medicine advanced therapy", "regulatory", "Europe" or "clinical trial". For regulatory requirements, the information available on EMA website and https://eur-lex.europa.eu/ was reviewed for matching legal acts or guidelines. The review of the applicable legal acts was performed based on knowledge gained from publications found during the literature review.

Results and Discussion

Within the European Union, drugs, medical devices and other medical therapies are classified into 3 main groups:

(1) medicinal products
(2) medical devices
(3) advanced therapy medicinal products

The classification of medical methods and therapies in Europe is based on the rule from the general to the more detailed. This method makes it possible to group therapies and select smaller specific subgroups such as gene therapies, somatic cell therapies, tissue engineering therapies and combination products within the ATMP group, or vaccines, antibodies, drugs within medicinal products. The manufacturer's doubts can be addressed through two pathways: the scientific recommendation procedure and the scientific advice procedure. These two pathways allow to discuss the requirements for a therapy before submitting a marketing application. If the manufacturer is interested, it can extend consultations with authorizing bodies in the USA via a parallel scientific advice session. Currently, there are 18 approved advanced therapies on the market, and the ratio between the submitted applications for scientific recommendation and the products available on the market is huge (FIG.1).

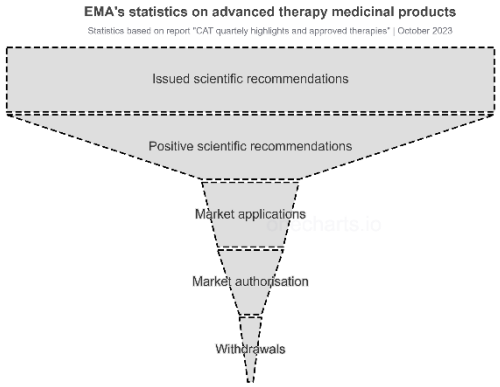

FIG. 1. Statistics on ATMP from European Medicines Agency.

The process of obtaining market authorization from the time a complete application is submitted takes up to 277 days, if the applicant is not called for additional supplements [3]. The Committee for Medicinal Products for Human Use (CHMP) issues a scientific opinion stating whether the product can be authorized and transmits it to the European Commission. However, the whole process can take much longer than a year. EMA uses the support of advisory committees or specialists. Each consultation lengthens the marketing authorisation process and can take anywhere from a few months to several years, depending on the complexity of the therapy, the amount of data available and any questions or concerns that may arise during the evaluation process.

Conclusions

In Europe, the regulations provide a fairly intuitive and unambiguous guide for an entity seeking product classification. When in doubt about the correct classification, it is worth using the forms of consultation provided by EMA. To summarize the most pertinent categories for regenerative medicine therapies are advanced therapy medical products.

Acknowledgements

Authors should acknowledge any person, or funding agency that has made a significant contribution to the work.

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STUDIES ON BIOCOMPATIBILITY AND PERMEABILITY OF NANOCOMPOSITE DIALYSIS MEMBRANES MODIFIED WITH CARBON NANOFORMS

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Introduction

One of the important parameters, which determine the possibility of using membranes in renal replacement therapy (RRT), is their selective permeability. To better understand the membrane's performance potential and predict how it will perform in vivo during RRT, its flux and selectivity need to be defined. Modern membranes must show effective removal of both small and large toxin, while restricting leakage of albumins, but the difficult task is to remove the middle molecules (MM) as well (e.g. β2-microglobulin: 12 kDa) [1]. Studies show that it is more effective in high-flux than low-flux membranes and that they are especially advantageous in patients with low serum albumin (< or = 4 g/dl), which is recognized as a marker of more severe illness [2].
Previous studies on nanocomposite membranes have shown that the addition of carbon nanoforms (graphite/ graphene/ carbon nanotubes) significantly affect not only the membrane morphology (pore size and distribution), but also regulate its surface properties: surface energy, wettability and thrombogenicity. These properties are important for permeability processes related to the membrane (bio)fouling and the reduction of filtration efficiency with the duration of dialysis. At this stage of the work, the focus was on verifying the filtration efficiency measured as the permeability of a membrane subjected to a hydrodynamic pressure. It was shown that the pore size (depending on the type of carbon nanoadditive) and the skin-support layer ratio are quantities that cannot be solely used as guide in the selection of a highly permeable membrane. Pore morphology plays an important role in determining the particle size during the selective permeation.

Materials and Methods

Polysulfone nanocomposite membranes modified with carbon nanoforms 1-2% wt., ie. carbon nanotubes (CNT), graphene oxide (GO) and graphite (GR), were obtained in phase separation process. A series of permeability tests was performed using flat sheet nanocomposite membrane fixed in a chamber filtration equipment (FIG. 1).
Membrane permeability was measured by determining the analytical feed composition versus the filtrate composition. Solutions of 0.05% bovine albumin (60 kDa), chicken albumin (40 kDa) and inulin (5 kDa) were used in permeability studies. Protein quantification (albumin: BSA, CSA) was performed by Exton reaction (UV-Vis Shimadzu 1900i) at 445 nm. Inulin content in the filtrate/filter was determined by UV-Vis method: indirect determination of inulin hydrolysate after reaction with $Cu(OH)_2$ at 668 nm.

Cell viability was determined by the Vialight®Plus assay (BioAssay Kit, Lonza, Basel, Switzerland) complementarily to the cytotoxicity assay (Toxilight). Both assays were carried out according to the protocol. The morphology of cells contacted with fibrous materials was observed after cells were dehydrated in a series of alcoholic solutions (C_2H_5OH, 40-96%) and covered with a gold layer (10nm, Leticia SP 12). The experiments were carried out using a scanning electron microscope (NovaNANO SEM, FEI).

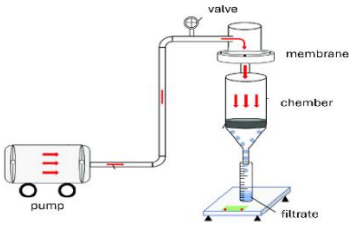

FIG. 1. Permeability filtration system.

Results and Discussion

The study showed that the permeability of nanocomposite membranes to BSA suspension is highest for GR and CNT membranes and is about 20%. Smaller CSA macromolecules are permeable at 20-30% for all nanocomposite membranes. The degree of permeability to inulin is the highest for CNT and GO membranes and is about 50% (FIG. 2).

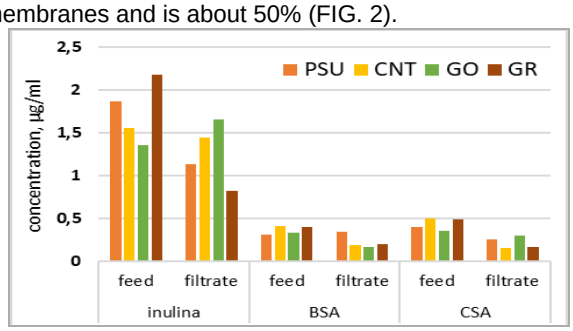

FIG. 2. Results of permeability test nanocomposite membranes.

The statistical analysis of the result of in vitro tests after 3rd and 7th day of material contact with RAW 247 human macrophage cultures showed high viability of the tested cells on the prepared membranes in comparison to unmodified samples. Statistically significantly higher viability was achieved for cells contacted with the surface of CNT and GR membranes. Nanocomposites membranes modified with carbon nanoforms, achieved better viability than polymer membrane PSU.

Conclusions

The presence of carbon nanoforms affects the permeability of nanocomposite membranes. The degree of permeability depends on the size of the separated particles. All membranes are biocompatible and thus have potential for biomedical applications as dialysis membranes

Acknowledgements

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NANOPARTICLE SIZE EFFECT AS A FACTOR INFLUENCING THE BIOMIMETICS OF SYNTHETIC HYDROXYAPATITE AND THE PROPERTIES OF IMPLANT COATINGS

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Introduction

The implant surface plays a key role in the body's biological response to the implant. Modification of the implant surface by applying hydroxyapatite coatings is used to accelerate the overgrowth of the implant with new bone tissue and to promote implant stabilisation. However, existing methods of depositing hydroxyapatite coatings have significant limitations. Taking advantage of the full potential of nanotechnology may provide a solution to these problems. Size effects at the nanoscale determine the properties of materials. Controlling these properties opens new possibilities for designing materials for bone tissue regeneration, such as regulating the degradation time and the amount of calcium Ca^{2+} ions released, and others.

Materials and Methods

Nanoparticles of nanohydroxyapatite GoHAPmed with precise controlled particle size were obtained using the hydrothermal microwave synthesis method described in detail by Kusnieruk et al. [1]. The fabricated nanoparticles were used in the process of coating the surfaces of titanium and polymeric implants by sonochemical method. The method of sonochemical deposition of hydroxyapatite layers was described by B. Wozniak, U. Szalaj, et al. [2]. Different particle size (10 nm, 14 nm, 26 nm, 30 nm, 42 nm) of obtained hydroxyapatite GoHAPmed were separately selected for coating.

Results and Discussion

This work concerns a unique eco-friendly microwave synthesis process enabling strict size control of hydroxyapatite nanoparticles (GoHAP™) in the range of 10±1 to 42±4 nm by controlling the synthesis parameters such as time, pressure and temperature. The complete characterization of GoHAP™ nanoparticles and the relationship between material properties and particle size has been demonstrated. This work presents the mechanism of formation of the nanoparticles layer deposited by sonocoating, as well as the relationship between the size of nanoparticles used in the coating process and the properties of the deposited layer. The presentation shows the kinetics of the nanoparticles layer deposition process depending on the GoHAP nanoparticles size as well as the properties of the obtained layers, such as morphology or contact angle, biocompatibility.

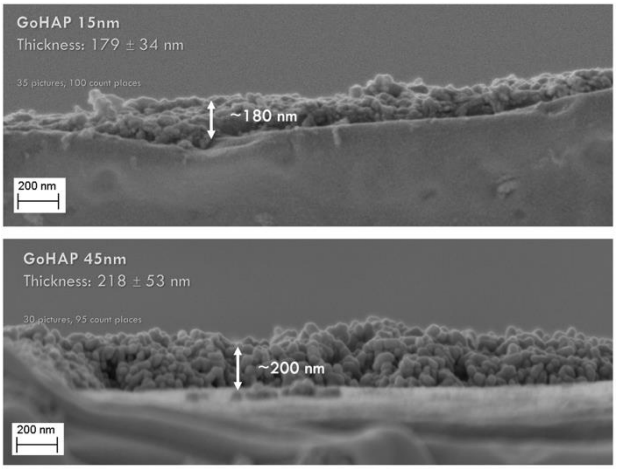

FIG. 1. Layer thickness investigations - SEM image analysis.

Conclusions

The study demonstrates that the size of the nanoparticles influences not only the properties of the nanomaterial, but also the similarity to hydroxyapatite naturally contained in bone. Furthermore, the advantages of ultrasonic deposition of hydroxyapatite nano-coatings as a universal deposition method for activity-programmed nano-coatings, unconstrained by the type, shape, or complex structure of the substrate to be coated, were demonstrated. This type of nano-coating deposition has been named the SonoNanoCoating (SNC) method. The SNC method also allows the full use of microwave hydrothermal nanoparticle synthesis (MHS) technology, in which GoHAPmed hydroxyapatite of adjustable particle size (10 nm, 14 nm, 26 nm, 30 nm, 42 nm) is obtained.
The SonoNanoCoating method overcomes the limitations of current implant surface coating methods by: I. Producing nano-coatings consisting of size-adjustable nanoparticles, thus enabling the adjustment of coating properties; II. Achieving homogeneous hydroxyapatite coatings on both the external surfaces and inside porous 3D structures and fibrous materials. III. Deposition of nano-coatings at low temperatures, enabling surface modification of temperature-sensitive materials IV. Exploiting the versatility of the method and selecting the parameters of nano-coating deposition independently of the type, shape and structure of the material being modified.

Acknowledgements

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APPLICATION OF 3D-BIOPRINTED LIVER TISSUE MODEL TO TEST THE EFFECTIVENESS OF TRADITIONAL AND ONCOLOGY PHARMACEUTICAL TREATMENT PROTOCOLS

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Introduction
Liver disease accounts for two million deaths each year and is associated with 4% of all deaths (1 in 25 deaths worldwide). Mortalities are mainly attributable to the complications of drug-induced liver damage and hepatocellular carcinoma. The drugs introduced into the patient's body are metabolized to a large degree by the liver. At present, it is a major challenge to plan effective therapy and validate pharmacological targets in patients requiring treatment particularly with drugs that are introduced as new to the commercial market. Certainly the lack of good preclinical models is an impediment in this regard. The models would enable the testing of drugs with hepatotoxic effects and used in cancer therapies. Particularly useful would be in liver cancer including HCC, which consequently implicates the human body. To date, animal models have often been used for drug testing, but they are not able to completely mimic the mechanisms in humans. New solutions are being explored to overcome these impasses as a result of the failures mentioned above. Development of 3D models of liver tissue in vitro has been proposed over the past few years. Models can be an alternative to preclinical studies performed on animals, completely depicting the mechanisms of drug metabolism and efficacy of therapies including cancer therapies in the human body. Appropriately designed 3D printed normal and cancer liver models will enable effective design of treatment protocols using traditional pharmacotherapy and oncology treatment protocols.

Materials and Methods
The study used models of normal and cancer liver tissue. The use of 3D-bioprinting and ink-jet technology required the development of bioinks that would be the optimal environment for the cells suspended in it. 3D-bioprinted models with a flow system was cultured for 21 days. Subsequently, drugs exhibiting hepatotoxic effects were administered to the channel in the case of the normal model, and anti-cancer drugs in the case of the cancer model. At the indicated time points after drug administration, material was collected in the form of biopsies and referred for histological analysis. Medium and tissue material were collected throughout the experiment to assess drug efficacy and functionality of the model. Analysis was performed using proliferation assays, lactic dehydrogenase release, immunoenzymatic assays and the expression of selected genes was assessed.

Results and Discussion
After administration of drugs demonstrating hepatotoxic effects, 3D-printed models of normal liver tissue showed an increase in the expression of CYP2A6 and CYP2C8, which are responsible for the metabolism of several pharmaceuticals. The highest fold change for CYP2A6 was observed after administration of rifamicin (RMP) at a concentration of 66 μM. Administration of lower concentrations of pharmaceuticals resulted in decreased expression of the gene CYP2A6 compared to control cells. The highest fold change of the gene CYP2C8 was observed after administration of RMP at 33 μM. The inhibition of cancer cell proliferation in the 3D-printed cancer liver tissue model was demonstrated after the administration of drugs exhibiting antitumour activity in the 3D-printed liver tissue model compared to the reference model after the administration of doxorubicin, a drug used in the oncological therapy of hepatocellular carcinoma. Inhibition of cell proliferation at a minimum of 50 % for the drug doses used. In addition, a dose-dependent decrease in alpha-fetoprotein concentration was observed in the culture medium.

Conclusions
The functionality and the stability of the 3D-printed liver model have been demonstrated on the studies. The developed technology will represent an alternate preclinical research pathway for testing new drugs.

Acknowledgements
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TWO ELPs PROTEINS DEVELOPED AS BIOINKS FOR 3D ORGAN BIOPRINTING

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Introduction

The aim of this project was to obtain recombinant structural proteins that, as a component of a bioink, would improve printing conditions and provide an optimal environment for cell colonisation and proliferation in the printed organ. Using genetic engineering techniques, we designed two different molecules based on the amino acid motifs that underlie the functional properties of elastin. To create proteins with elastomeric properties, we used multi-repeat motifs representing pentameric elastin sequences in combination with domains known from resilin and silk fibroin, and a fibronectin-derived sequence containing an RGD motif: RE15mR and EJ17zipR.

Materials and Methods

These proteins were obtained using a prokaryotic expression pT7 system induced by adding IPTG. The efficient fermentation process was performed in a fermenter in a LB medium volume of 3 l. The proteins were purified from bacterial cells in a multistep process including ammonium sulfate desalting or FPLC chromatography using Macro-Prep High Q Media resin (anionite). Peptide maps and molecular weights were confirmed by mass spectrometry and the purity of the samples obtained was examined by HPLC.
A series of basic rheological and 3D printing tests were carried out. At the same time, the effects of both proteins on cell adhesion and proliferation were evaluated in biological studies. Finally, the concentration of the proteins that would prevent a cytotoxic effect on the colonised cells was determined.

Results and Discussion

The proteins obtained were characterised by very high purity (98% according to HPLC) and stability when stored after lyophilisation. The proteins obtained were tested for mechanical, physico-chemical and biological properties. It was found that the addition of each of them improved the printing parameters, the fibres obtained maintained their continuity and showed low collapsing tendency. Increasing the concentration of recombinant proteins led to an increase in dynamic viscosity and storage modulus, giving the material more elastic properties than viscous ones, ensuring better printing resolution. Simultaneously, it was observed that the addition of the recombinant protein did not reduce print stability or adversely affect the shape of the construct. EJ17zipR, in particular, is characterised by its high flexibility, which, when using concentrations of 1 mg/ml, was manifested by its inability to be crushed when exposed to significant force, and the printed construct returned to its shape. Furthermore, in the presence of the RE15mR protein, a noticeable adhesion and proliferation of cells of a reference fibroblast line (L929) was observed. A similar but weaker effect was observed for EJ17zipR. Both proteins showed no cytotoxic effect in the cell viability assay, either in the classical MTT assay or in AlamarBlue or Calcein and EthD-1 staining.

Conclusions

As a result of the project, the biomaterial samples obtained maintained or enhanced the positive properties of the bioink used (plasticity, elasticity or strength), while increasing the level of cell attachment in the printed construct and creating a favourable microenvironment for their growth and proliferation. The resulting proteins are a potentially interesting ingredient for bio-inks. Their variable proportions in organ-specific compositions could modify the desired properties to mimic the characteristics of the natural organ.

Acknowledgements

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TITANIUM(IV) OXO-COMPLEXES WITH α-HYDROXY-CARBOXYLATE LIGANDS: THEIR STRUCTURE AND ANTIMICROBIAL PROPERTIES

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Introduction

Titanium(IV) oxo complexes (TOCs) are known for structural diversity and photocatalytic activity [1]. It is possible to obtain TOCs with cores containing from 2 to 44 titanium atoms [2]. Usually $\{Ti_aO_b\}$ cores are stabilized by alkoxide groups as well as carboxylate, phosphonate, β-diketonate, β-ketoester, and sulfonate ligands [3]. The use of α-hydroxy acids (α-HA) as stabilizing factor seems to be interesting because of increased coordination possibilities with Ti(IV) and from biological point of view due to their anti-inflammatory and antimicrobial properties.

The aim of research was the synthesis of TOCs with a core stabilized by α-hydroxy carboxylates as well as spectroscopic characterization of the obtained compounds, preparation of composite materials and testing their antibacterial properties.

Materials and Methods

Titanium(IV) oxo-complexes (FIG. 1 and FIG. 2) were obtained under an inert atmosphere, by mixing titanium(IV) isopropoxide with 9-hydroxy-9-fluorenecarboxylic and propionic acid. Composite materials were obtained by introducing 2%, 5%, 10%, 20% TOCs into a poly(methyl methacrylate) (PMMA) matrix. Microbiological tests of composites were carried out for the following bacteria: *Escherichia coli, Staphylococcus aureus* and *Candida albicans* yeasts. MTT tests conducted on the L929 murine fibroblast cell line were carried out to estimate cytotoxicity.

Results and Discussion

Our results confirmed that the reaction of titanium alkoxides with α-hydroxy acids allows obtaining TOCs whose core is stabilized by both the interactions of the carboxylate and the hydroxyl group. Two mechanisms of the microbiological action of TOCs are postulated: (i) related to the photocatalytic activity of the oxo complex and (ii) caused by structural factors, especially the formation of chelate rings. The obtained materials do not exhibit cytotoxicity.

Conclusions

These compounds and their composites can be utilized as an antibacterial coating in public facilities or hospitals to mitigate the risk of bacterial infections.

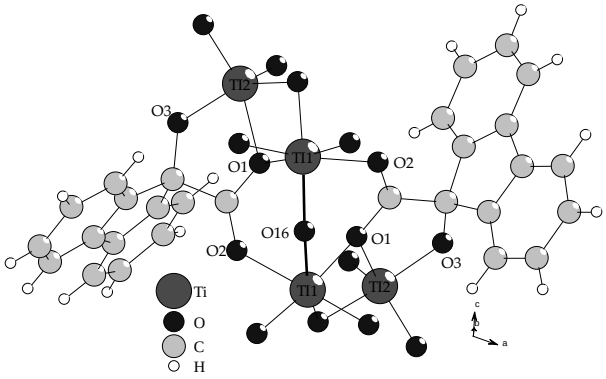

FIG. 1. Structure of $[Ti_4O(O^iPr)10(O_3C_{14}H_8)_2]$ as a ball and stick model.

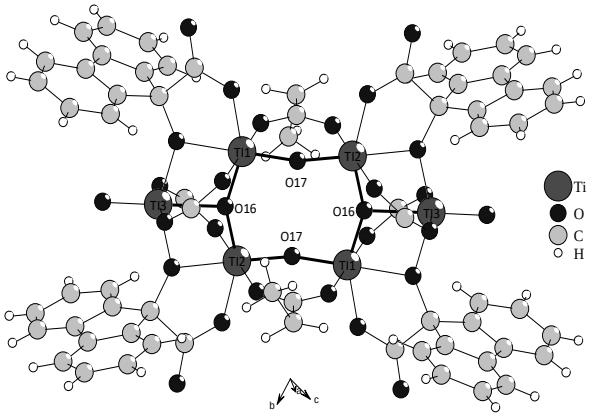

FIG. 2. Structure of $[Ti_6O_4(O^iPr)_2(O_3C_{14}H_8)_4(O_2CEt)_6]$ as a ball and stick model.

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ENHANCING BIODEGRADABLE 3D IRON SCAFFOLDS: HYDROXYAPATITE SYNTHESIS VIA CATHODIC ELECTRODEPOSITION

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Introduction

The necessity of using biodegradable materials in implants stems from the need to minimize long-term complications associated with the presence of foreign bodies in the body. Biodegradable implants are gradually broken down by the body, eliminating the need for their removal through additional surgeries. Furthermore, such implants can be designed to degrade at an appropriate rate, supporting the natural healing and regeneration process of bone tissue. In order to improve the biointegration of bone implants, hydroxyapatite is used, this as a crucial material used in bone implants due to its unique biological and chemical properties that closely resemble natural bone tissue. Its biocompatibility, bioactivity, and ability to integrate with natural bone make it an ideal material for supporting bone regeneration. Hydroxyapatite accelerates osteointegration, leading to better stability and durability of bone implants. Using biodegradable materials in combination with hydroxyapatite in bone implants allows for the creation of advanced medical solutions that could be safer, more effective, and better aligned with the natural biological processes occurring in the human body - that is our goal.

Materials and Methods

The macro-porous Fe scaffolds were made of iron powders particle sizes <10 micron. The samples prepared in this way were placed in a tube furnace and sintered for 5h in II steps (I step from RT to 500°C with rate 8°C/minute, II step to 1050°C with rate 6°C/minute). Hydroxyapatite on the surface of biodegradable iron scaffolds was synthesised using the cathodic electrodeposition method using three different current intensities (0.5mA, 1.0mA and 1.5mA). The morphology of the produced scaffolds was studied using a Quanta scanning electron microscope with field emission. Corrosion behaviour was determined using immersion and potentiodynamic polarization methods in phosphate buffered saline (PBS). The surface energy was calculated by studying the changes of enthalpy of calorimetric immersion.

Results and Discussion

The hydroxyapatite layers were successfully synthesized on biodegradable iron scaffolds using cathodic electrodeposition at current intensities of 0.5mA, 1.0mA, and 1.5mA, with SEM analysis revealing uniform and consistent coating morphologies. The hydroxyapatites produced at three different current intensities differ in their morphology and calcium:phosphorus ratio. In natural bone, the Ca:P ratio is 1:67, the nearest value to this is the hydroxyapatite produced at 0.5mA. Corrosion tests in phosphate-buffered saline demonstrated that the hydroxyapatite-coated scaffolds experienced slightly accelerated corrosion, which is beneficial for controlled degradation and gradual replacement by natural bone tissue.

Conclusions

These results highlight the potential of combining hydroxyapatite with biodegradable iron scaffolds to create advanced bone implants that promote natural healing while ensuring degradation of implants and replacement by new bone tissue.

Acknowledgements

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ABDUCTIN AS A NOVEL POTENTIAL BIOMATERIAL COMPONENT

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Introduction

One of the challenges standing in front of the constantly expanding field of tissue engineering is the requirement of biomaterials that, in addition to pro-cellular functionality, also display mechanical features that resemble the native tissue. Precise deposition of cell-laden material in three-dimensional structures facilitated by 3D bioprinting technology has exacerbated the need for novel biomaterials to help retain the structure of openwork tissue-mimicking scaffolds. Elastomeric proteins have been intensively studied for such applications due to their distinct mechanical properties which result from domain-repetitive structure and crosslinking ability. Studies on the modular nature of elastomeric proteins, such as elastin, resilin, or spider silk, emerged in numerous works, investigating their recombinant analogs. In the 1960s, the literature described abductin, a small protein found in molluscs, whose mechanical parameters exceed those of elastin or resilin [1-4]. To date, only one study concerning abductin-based protein usage has been published (in 2013) denoting promising results [5]. To our knowledge, this is the first attempt to produce full-length abductin and assess its biological and mechanical properties.

Materials and Methods

To produce abductin in a prokaryotic system, a DNA template encoding an *E. coli*-optimized amino acid sequence of abductin was prepared. Subsequently, using a set of primers and multiple PCR reactions, an open reading frame encoding full-length abductin has been obtained. Additionally, one of two secretion signals (from bacterial genes encoding the maltose-binding protein (malE) or beta-lactamase (bla)) was fused at the N-terminal end for extracellular protein transportation. To improve protein detection and purification, HIS-tag domain sequence was added at C-terminal end of abductin. The DNA templates were cloned into the pGEX-6P expression vector and introduced into *E. coli*, where the expression of abductin was induced using IPTG.

Results and Discussion

To improve protein production, two of three designed open reading frames encoded derived from malE or bla bacterial genes additional secretion signal peptide at the N-terminal end of abductin. The third vector encoded abductin without secretion signal. All proteins have been produced in a modified *E. Coli* strain overexpressing several specific tRNAs enabling the synthesis of proteins with enriched glycine content. Despite additional secretory peptide attached to the N-terminus, both proteins were present in bacterial pellets but not in the culture medium. Surprisingly, the abductin variant lacking signal peptide was not detected with western blot (FIG. 1). Purified abductin was incubated with functionalized superparamagnetic iron oxide nanoparticles (SPIONs) with high affinity to HIS-tag sequence. As a result, efficient formation of abductin-SPION complexes has been observed.

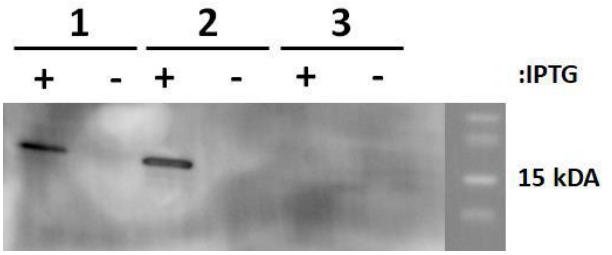

FIG. 1. Western blot analysis of IPTG-induced bacterial extracts. 1 – abductin variant with signal peptide derived from bla protein; 2 - abductin variant with signal peptide derived from malE protein; 3 - abductin variant without signal peptide.

Conclusions

The literature describes abductin as a protein with remarkable mechanical properties. However, for unknown reasons, abductin has not been further studied in terms of biomaterial and its application potential remains unexplored. Hence, we produced a full-length protein *in cellulo* and subjected it as a potential new compound in biomaterial sciences.

Acknowledgements

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THE COMPREHENSIVE STUDY ON LACTOFERRIN-RUTHENIUM COMPLEXES: METHODOLOGY OF PREPARATION, BINDING MECHANISM, AND BIOLOGICAL PROPERTIES

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Introduction

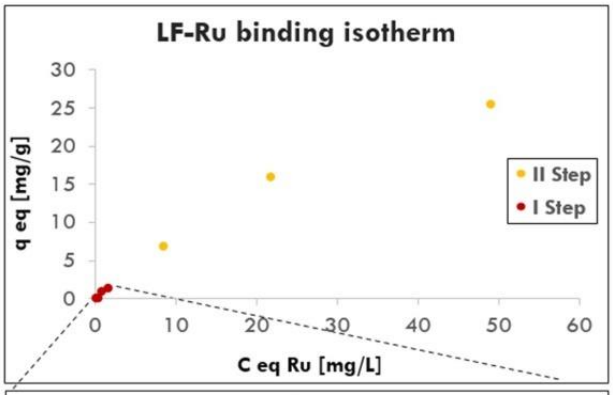
Bovine lactoferrin (bLF), a well-known multifunctional glycoprotein, which exhibits a strong binding affinity for ferric (III) ions (K_D 10^{-20} M) [1]. For this reason, it acts as an iron carrier for various cell types, including immune and intestinal epithelial cells [2]. Thanks to its unique structure, bLF is capable to bind various metals, such as zinc (II), copper (II), chromium (III), manganese (III), cobalt (III), and aluminum (III) [3]. Despite the evident anticancer activity, ruthenium (Ru) compounds have garnered significant interest in recent years due to their promising antimicrobial properties. These compounds have been shown to possess broad-spectrum activity against various pathogens, including bacteria, fungi, and viruses [4]. The development of metal-protein complexes represents an innovative approach aimed at enhancing the existing properties of proteins and creating biomolecules with expanded functionality. Therefore, the major goal of this research relies on the development of methodology for the preparation and LF-Ru complexes, as the potential antimicrobial agents.

Materials and Methods

The preliminary synthesis of LF-Ru complexes was performed for 24 hat pH 4 and 37°C using the wide range of Ru concentrations from 0.4 to 123.7 mg/L. The aquapentachlororuthenate(III) was used as a ruthenium precursor. In addition, the kinetic studies on the LF-Ru complex formation were performed at t (min) 1, 5, 15, 30, 45, 60, 120, 300, 450, 600, 1440. After the incubation, the non-bounded fraction of Ru(III) was dissolved in 2% HCl and the metal concentration was determined by ICP-OES. The conformational changes and the stability of the prepared LF-Ru compounds was characterized by series of instrumental techniques, such as UV-Vis, spectrofluorimetry, and SDS-PAGE. Finally, the antimicrobial activity of LF-Ru complexes was evaluated against *S. aureus* and *E. coli* strains.

Results and Discussion

The obtained binding isotherm indicates that the formation of the LF-Ru complex occurs in two distinct steps (FIG. 1). The initial step involves weak sorption processes at low metal concentrations, described by the Henry model. The second step corresponds to the formation of a homogeneous monolayer, as described by the Langmuir model. ICP-OES results showed a relatively high sorption efficiency of Ru(III) to bLF, ranging from 7.1% to 36.9%. The highest binding percentage of ruthenium was observed at an initial Ru(III) concentration of 2.6 mg/L. As the metal concentration increased further, the sorption efficiency gradually decreased, indicating a desaturation effect. The significant quenching of fluorescence intensity (from 9100 a.u. to 4000 a.u.) confirms the binding of Ru(III) to bLF. Additionally, the observed red shift in the fluorescence maximum from 328 nm to 332 nm after LF-Ru complex formation may be due to partial unfolding of the bLF structure.



Freundlich model			Langmuir model		
n	K_F [mg/g]	R^2	q_{max} [mg/g]	K_L [mg/g]	R^2
1.38	1.56	0.992	54.5	0.02	0.999

FIG. 1. The binding isotherm of LF-Ru performed at pH 4.

Conclusions

The proposed synthesis method ensured a relatively high sorption efficiency in LF-Ru systems. This phenomenon might be explained by the ability of Ru(III) to mimic Fe(III). Spectroscopic studies, indicated that complex formation accompanied with a significant change in the three-dimensional structure of bLF molecule. The current study confirmed the high antibacterial potential of the prepared LF-Ru complexes. As it is known, both LF and Ru(III) exhibit strong antibacterial properties, thus this might be related to their synergistic effect.

Acknowledgements

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UNDERSTANDING THE EFFECT OF ULTRAFILTRATION ON THE PHYSICOCHEMICAL AND STRUCTURAL ASPECTS OF BOVINE LACTOFERRIN

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Introduction

Bovine lactoferrin (bLF) is widely recognized as iron-binding glycoprotein, which shows a wide spectrum of biological activity, including antibacterial, antiviral, antioxidant, immunomodulatory, and anticancer functions [1]. Despite, the protein attractiveness as a promising nutrient and therapeutic agent, the widespread application of bLF is restricted due to trace protein concentration in the potential sources, such as mature milk (0.02-0.2 mg/mL) and colostrum (1-5 mg/mL) [2]. bLF isolation is described as multi-stage complicated procedure, which in practice accompanies with application of great amounts of concentrated salt buffers. For this reason, salts are recognized as one of the major protein impurities. In addition to it, the inappropriate storage conditions might promote the degradation effects in the protein structure. The mentioned factors might significantly reduce the quality of the final product. In order to eliminate, the present salts as well as possible degradation products, the relevant pre-treatment procedure is required. Ultrafiltration (UF) is a popular membrane technique, which is widely applied for the protein concentration and desalting [3]. As it is expected, the more precise bLF purification could improve the overall sample representation. This study is aimed to evaluate the efficiency of UF on the bLF purification. Besides, the structural modifications as well as changes in the molecular masses, sequences, and post-translational modifications of both bLF samples, before and after UF, were compared.

Materials and Methods

In this study, the two types solvents were tested during UF: ultrapure water and saline. The protein purification efficiency was monitored by SDS-PAGE. The role of UF on the structural modifications in bLF was analyzed by microscopy studies. The accurate molecular mass, primary structure (sequence), and changes in glycosylation heterogeneity of lactoferrin, were analyzed using MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) techniques. The mass profile of the intact bovine lactoferrin was analyzed by application of saturated sinapinic acid as a matrix and the Protein Calibration Standard II as a calibrant. In-gel tryptic digestion was performed for the protein identification. Saturated α-cyano-4-hydroxycinnamic acid and the Peptide Calibration Standard II were used as the matrix and calibrant, for the analysis of extracted peptides. Initially, the sample was mixed with the matrix solution in a 1:1 (v/v) ratio and then applied to a Ground Steel target plate using the dried-droplet method. The peptide mass fingerprint spectra were monitored in reflectron positive ion mode within an m/z range of 500-3500. Cysteine carbamidomethylation and methionine oxidation were selected as global and variable modifications, respectively. The Mascot database, along with BioTools, was used for result interpretation.

Results and Discussion

It was determined that initial dissolving of bLF in saline facilitated the effective protein purification by removing smaller fragments, thereby reducing membrane pore clogging. The results indicated no distinct changes in the molecular masses between protein subjected and non-subjected to UF, which in both cases was at around 82-83 kDa. Microscopy studies revealed a noticeable transformation in the bLF surface structure after UF, changing from smooth to more porous and rugged (FIG. 1). According to MALDI-TOF-MS results, four potential glycosylation sites were indicated at Asn300, Asn349, Asn537, and Asn640 in case of both bLF, before and after UF. Additionally, the improvement of identification parameters, such as sequence coverage (from 52.7% to 61.6%) and intensity coverage (from 84.5% to 89.2%), indicate the positive effect of UF on the sample representation.

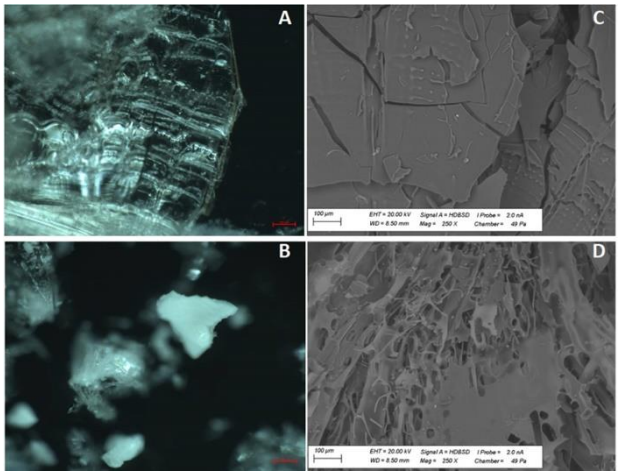

FIG. 1. Light microscopy (A,B) and Scanning electron microscopy images (C,D) of bovine lactoferrin before (A,C) and after ultrafiltration (B,D).

Conclusions

The large-scale isolation of bovine lactoferrin (bLF) typically involves using large amounts of concentrated eluents, which might introduce impurities to the final product. Sometimes, protein pre-concentration is required for the greater accuracy of experimental results. In this research, the supplied bLF sample was subjected to additional ultrafiltration (UF) to eliminate possible small impurities, such as salts and peptides of bLF. Our results showed, that the prior UF of bLF allowed for the efficient protein purification. Besides, the improvement of identification parameters, confirms the importance of UF procedure as an essential pretreatment step, leading to higher accuracy of results during the protein analysis.

Acknowledgements

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ONE STEP CLOSER TO MENISCUS REGENERATION: MENISCAL dECM BASED BIOINK FOR SCAFFOLD 3D BIOPRINTING

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Introduction

A rapidly growing field of regenerative medicine offers the possibility to restore the physiological functions by implementing state-of-the-art tissue engineering technique - 3D bioprinting. Decellularized extracellular matrix (dECM) is an excellent bioink component, closely mimicking native tissue environment including structural and biomolecular properties. 3D bioprinting of meniscus implants creates the opportunity to improve the long-term effects of meniscal injuries treatment. However, this tissue being the hard and elastic one, presents significant processing challenges, making most of the existing procedures of dECM extraction ineffective. In this study, an innovative, detergent-free approach to bioink formulation from decellularized porcine meniscus ECM has been proposed.

Materials and Methods

In this study, we propose a novel method for bioink formulation utilizing dECM obtained from porcine menisci, specifically designed for the 3D bioprinting of meniscal implants [1]. The decellularization protocol involves a set of extraction and digestion steps, including supercritical CO_2 extraction. The rheological properties of various bioink compositions containing dECM, alginate, gelatine, were evaluated. Selected bioinks were used for printing accuracy measurement and SEM imaging. For the biological study, human adipose-derived mesenchymal stem cells (hMSC-AT) cultured in monolayer or spheroids were mixed with bioink and 3D bioprinted into constructs. The cell viability within the constructs was analyzed using LIVE/DEAD assay at 1, 10, 20, and 30 days post bioprinting. At the same time intervals, RT-qPCR analysis of selected genes coding: ECM components (COL1A1, COL6A1, COL10A1 and COMP), transcription factors involved in chondrogenesis (HIF1A, RUNX2, SOX5, SOX9, SMAD2) and N-cadherin (CDH2), which plays a role in cell to cell adhesion during MSC condensation and transformation were performed.

Results and Discussion

Collected data revealed a high efficiency of the decellularization process which resulted in preservation of pivotal ECM structural components (collagen, GAG) with simultaneous removal of donor DNA material. Our protocol retained a substantial amount of ECM components – 92.2% of collagen and 59.9% of GAGs. Collagen amount is comparable with other protocols, while retained GAG content is substantially higher [2]. Since it has been established that GAGs play an important role in maintaining the compressive stiffness and load-bearing capabilities of articular cartilage, the need for maximal GAGs retention in the decellularization process is evident. Rheological analysis indicated that the bioinks are viscoelastic materials with a gel-like or solid structure and are appropriate for the use in 3D bioprinting procedure. High cell survival within 3D bioprinted constructs and significant changes in the expression level of chondrogenic markers have been observed proving dECM-base bioink biocompatibility and suggesting its positive influence on chondrogenic differentiation of MSC.

Conclusions

Taken together, this study provides a description of meniscal dECM production and formulation of dECM-based bioink suitable for meniscus tissue engineering.

Acknowledgements

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ANTIBACTERIAL SURFACES: FUNCTIONALIZATION OF POLYPROPYLENE WITH NANO TiO_2

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Introduction

Biomaterial-associated infections are common and feared complications in medical practice, usually resulting from the attachment of microorganisms to the surface of medical devices. There are caused by varieties of bacteria, both Gram-negative and Gram-positive, making it difficult to develop a universal treatment. In addition, the formation of biofilm on the surfaces of medical devices leads to antibiotic resistance. There are several ways to prevent biomaterial-associated infections, including the deposition of nanoparticles on the surface of the medical devices [1]. Medical tools and accessories are often made from polymers, e.g., medical aprons, syringes and cannulas made from polypropylene (PP). Polypropylene is widely available, inexpensive and exhibits remarkable chemical stability and hydrophobic characteristics, making it challenging to modify the surface to achieve specific functionalities. The use of low-temperature oxygen plasma enables the generation of surface functional groups resulting in improve material adhesion and facilitates the efficient deposition of nanoparticles onto the polypropylene surface [2]. Among many nanoparticles exhibiting antibacterial properties, the deposition of titanium oxide (TiO_2) nanoparticles on the PP surface may lead, upon irradiation, to generation of reactive oxygen species (ROS) that exhibit antibacterial properties (FIG. 1). TiO_2 is distinguished by its high photoactivity, non-toxicity, and chemical stability [3]. The aim of this work was to develop a synthesis method for the deposition of commercially available and photoactive TiO_2 nanoparticles over a polypropylene substrate to provide antibacterial properties.

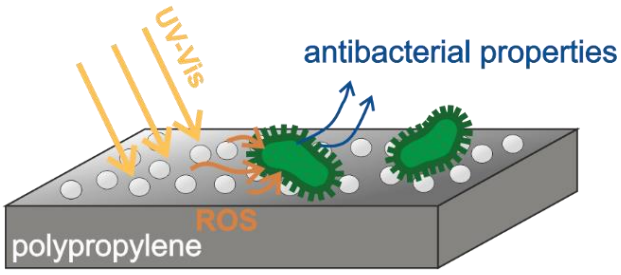

FIG. 1. Graphical scheme demonstrating the antibacterial properties of TiO_2/PP nanocomposite.

Materials and Methods

Commercially available components: TiO_2 nanoparticles (Cinkarna Celje d. d.) and polypropylene sheet (Merck) were used in the investigation. For optimization of the TiO_2/PP nanocomposites performance the parameters of plasma treatment (time, pressure) and the conditions of sonochemical deposition (time, amplitude, nanoparticle concentration) were adjusted. Effect of plasma modifications was investigated using surface contact angle (WCA) measurements, and the formation of functional groups on the surface was checked using Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The size of the nanoparticles was examined using nanoparticle tracking analysis (NTA) and microscopic methods (TEM, SEM), while their arrangement on the polymer surface was examined using scanning electron microscopy (SEM) and atomic force microscopy (AFM). To investigate the generation of reactive oxygen species by nanocomposites upon irradiation, tests using a hydroxyl radical scavenger - terephthalic acid (TA) were conducted. Antibacterial properties of TiO_2/PP nanocomposite have been determined in bacteria adhesion tests using Gram-positive and Gram-negative bacteria strains.

Results and Discussion

TiO_2 nanoparticles were smaller than 10 nm in size, with a tendency to form agglomerates of typically around 50 nm. The efficiency of nanoparticle deposition over polypropylene surface increases significantly after surface modification with oxygen plasma treatment. Generation of functional groups was confirmed due to the presence of mainly hydroxyl, carbonyl, and carboxyl groups (-OH, -C=O, O-C=O) on the surface of polypropylene upon plasma treatment. It was found that the optimized parameters of plasma treatment and sonochemical deposition are of key importance for antibacterial properties of the final nTiO_2/PP nanocomposite. The antibacterial effect was discussed in terms of ROS generation during UV-Vis irradiation and their interactions with the bacterial cell walls.

Conclusions

The obtained results showed that by optimizing parameters of both plasma treatment and sonochemical deposition the surface concentration and the accessibility of the photocatalysts surface can be controlled. The TiO_2/PP nanocomposites generate reactive oxygen species upon irradiation and have antibacterial properties. Modifying polypropylene with titanium nanoparticles and thus adding antibacterial properties, makes it beneficial for biomedical applications. The universality of the developed modification pathway allows the use of a wide range of polymers and nanoparticles, and tailor their properties to specific requirements.

Acknowledgements

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MODIFIED COATED FABRIC FOR MEDICAL AND VETERINARY APPLICATIONS

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Introduction

Coated fabrics are materials with a wide range of applications. Through appropriate design and modifications, we can impart desired properties, enabling targeted use. This approach aims to enhance functional qualities, thereby improving the quality of life. In medicine and veterinary science, coated fabrics can significantly increase the levels of hygiene and protection of health and life.

In the production of coated fabrics, antibacterial additives are most commonly based on silver [1]. However, silver ions exhibit cytotoxic properties, which naturally leads to the search for alternative solutions. Betulin can serve as a modifier for coated fabrics owing to its antibacterial, antiviral, and anti-inflammatory properties. Polymers modified with betulin exhibit antibacterial effectiveness against Escherichia coli DSM 1576 and Staphylococcus aureus DSM 346 with an efficacy level of R<1, even at concentrations below 0.1% [2, 3]. This suggests that incorporating natural betulin into coated fabrics can provide substantial benefits in medicine and veterinary applications.

Considering the cytotoxic risks associated with silver and the natural sourcing of betulin, adopting such modified fabrics for widespread use in medicine and veterinary science promises not only to enhance the efficacy of health protection but also to promote environmental sustainability by substituting silver with a renewable material.

Materials and Methods

This study investigates the modification of a polyester fabric using a plastisol mixture (PVC combined with plasticizer and various modifiers) that incorporates 1% betulin. The betulin was extracted from birch bark using the Soxhlet extraction method and subsequently combined with plastisol through a plasticizer-based premix to ensure compatibility and thorough homogenization. The resulting mixture was applied to the fabric using blade coating technology under laboratory conditions. The antibacterial properties of the modified fabric were assessed according to the ISO 22196:2007(E) standard, utilizing Escherichia coli DSM 1576 and Staphylococcus aureus DSM 346 as test organisms. Additionally, tribological tests were conducted to evaluate the impact of the betulin modification on the surface properties of the coated fabric. These tests included measurements of the average coefficient of friction using a standard tribometer. The findings indicate that betulin shows significant potential as an alternative to traditional silver-based modifiers for enhancing the antimicrobial resistance of PVC-coated textiles, offering a promising avenue for future textile innovations.

Results and Discussion

The study conducted following ISO 22196:2007(E) "Plastics - Measurement of antibacterial activity on plastic surfaces" demonstrates that betulin reduces the number of viable bacteria of Escherichia coli DSM 1576 and Staphylococcus aureus DSM 346. A moderate antibacterial effect of betulin against both tested strains was observed. Reduction values (R) fall within the range indicating acceptable antibacterial activity [4].

Preliminary tribological tests suggest that the addition of betulin may positively influence the coefficient of friction, potentially enhancing the durability of the coated fabric [4].

The observed antibacterial activity in betulin-modified fabric underscores the potential of natural compounds as alternatives to traditional antibacterial agents such as silver. Additionally, tribological tests suggest that betulin may improve the surface properties of PVC-coated textiles. This suggests that modifications with natural extracts could be a promising approach to creating multifunctional textiles with enhanced antibacterial and tribological properties.

Conclusions

Betulin, as a textile-modifying agent, shows significant potential in both medicine and veterinary science. Research has confirmed its moderate yet notable antibacterial effect against Escherichia coli and Staphylococcus aureus, suggesting its value as an alternative to traditional agents like silver. Additionally, betulin enhances the tribological properties of materials, which can lead to prolonged durability of textile coatings.

In the medical field, betulin can be used to produce protective clothing, dressings, curtains, and disinfectant materials, providing additional protection against hospital-acquired infections and improving hygiene during rehabilitation. In veterinary medicine, textiles modified with betulin can be employed in making pads, mattress covers, and protective clothing for animals, thereby enhancing safety and comfort during veterinary procedures and transportation.

Further research on betulin could lead to innovative solutions in biomedical materials, contributing to improved hygiene and safety standards for both humans and animals.

Acknowledgements

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SUPERSTRONG ALGINATE/ POLYVINYL ALCOHOL HYDROGELS MODIFIED BY CROSSLINKING MECHANISM

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Introduction

Hydrogels are characterized by extremely attractive properties for biomedical applications, such as high biocompatibility, similarity to the extracellular matrix (ECM), ease of formation, and shaping of biological properties [1]. However, for many applications, their low mechanical strength is a limitation. In the scientific literature, numerous papers on the modification of the properties of hydrogels to improve their mechanical parameters can be found, but the compounds used for this purpose are most often toxic [2].
The aim of the study was to obtain superstrong alginate/polyvinyl alcohol double network hydrogels by using cross-linking methods based on non-toxic compounds for use in filling osteochondral defects. In the presented research, the effect of different cross-linking methods on the mechanical properties of hydrogels was evaluated.

Materials and Methods

The following reagents were used in the fabrication of scaffolds: sodium alginate (Acros); polyvinyl alcohol (PVA) (Mw approx. 145000, Merc); $CaCl_2$ (Sigma Aldrich); $FeCl_3 \cdot 6H_2O$ (POCH); 6M NaOH (Chempur).
In the first step, hydrogel solutions were prepared: 5% alginate (dissolved in deionized water at 50°C on magnetic stirrer); 5% PVA (dissolved in deionized water at 90°C on magnetic stirrer). Next, the alginate and PVA solutions were combined 1:1, homogenized at 50°C by 30 min on magnetic stirrer, molded in the cylindrical form (10 mm in diameter x 9 mm in high), frozen (30 min at -20°C, next 24h at -80°C) and freeze-dried (48h in LABCONCO freeze-dryer). Next, the samples were cross-linked. The following approaches were used in the cross-linking process: cross-linking in freeze/thaw cycles (18h at -20°C, 6h at 25°C, 3 times repeated), then in a 1% solution of $CaCl_2$ by 4h **(1) – Alg/PVA**; cross-linking in freeze/thaw cycles (as described above), then in a solution of 1% $CaCl_2$ and 0.5% $FeCl_3$ by 4h **(2) – Alg/PVA-Fe**; cross-linking in 1% $CaCl_2$ (by 4h) followed by etching in 6M NaOH (15 min.) **(3) – Alg/PVA-NaOH**. Comparatively, the alginate cross-linked in 1% $CaCl_2$ (by 4h) **(4) - Alg** and in a solution of 1% $CaCl_2$ and 0.5% $FeCl_3$ (by 4h) **(5) – Alg-Fe** were obtained (samples casting from 2.5% alginate solution). After cross-linking, the samples were rinsed in distilled water, frozen (24h at -80°C) and freeze-dried (48h).
The mechanical tests were conducted using the Zwick 1435 universal testing machine. The compression modulus (Ec) and the compressive stress at 50% relative contraction $\sigma_{(\varepsilon=50\%)}$ were determined in a static uniaxial compression test. The porosity of the samples was determined in ethanol [3]. Samples were triplicate. All data were calculated using the mean ± standard deviation. Differential scanning calorimetry (DSC) was performed under a protective atmosphere (nitrogen), 10°C/min, from -10°C to 350°C, (Star software, Mettler Toledo DSC1).

Results and Discussion

For Alg/PVA treated in NaOH, the highest value of compression modulus (1.5 MPa) and stress (6.4 MPa) was observed (FIG. 1). It can be related to microstructural parameters (the lowest porosity equals 11%) but also with higher crystallinity (FIG. 2). This material had also the highest thermal stability. Cross-linking in $CaCl_2$+$FeCl_3$ caused only slight changes in mechanical parameters of Alg/PVA-Fe compared to Alg/PVA cross-linked in $CaCl_2$, however, increased significantly porosity (55% and 20%, respectively). The presence of Fe^{3+} ions in the hydrogel structure significantly increased the crystallinity and thermal stability of PVA (FIG. 2). Alginate showed the highest porosity (78% for Alg and 74% for Alg-Fe) and mechanical parameters significantly lower than Alg/PVA, which indicates a substantial impact of the double polymer network regardless of the cross-linking method used.

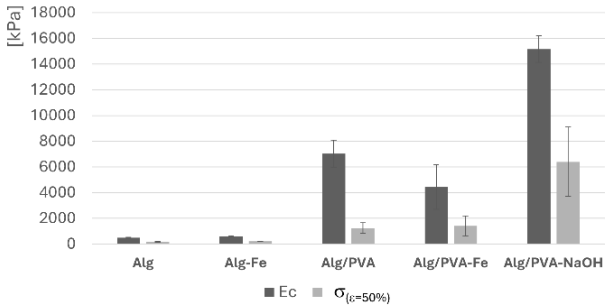

FIG. 1. Compression modulus (Ec) and compression stress (σ (ε=50%)) of the hydrogels.

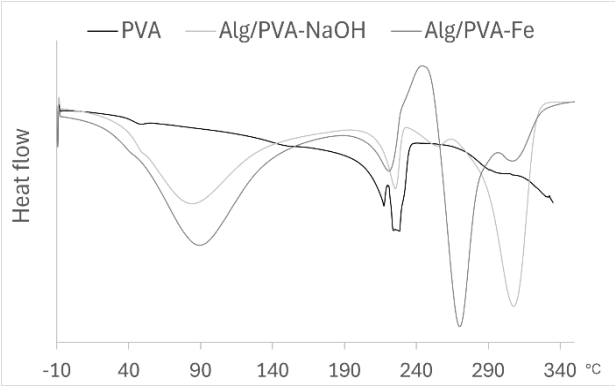

FIG. 2. DSC curves of hydrogels.

Conclusions

The highest strength of 6.4 MPa was obtained for alginate/PVA cross-linked in 6M NaOH.
The alginate/PVA hydrogel had significantly higher mechanical parameters compared with those of alginate alone, which confirms the purposefulness of using double-network hydrogels. The use of $FeCl_3$ in a cross-linking solution only slightly increased the strength of alginate and alginate/polyvinyl alcohol. Observed changes resulted from microstructural and structural properties of hydrogels.

Acknowledgements

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REVEALING THE POTENTIAL OF NUCLEAR MAGNETIC RESONANCE IN CHARACTERIZATION AND SUSTAINABLE DEVELOPMENT OF BIOMATERIALS

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Nuclear Magnetic Resonance is a one of the most powerful and commonly used technique in the fields related to chemical, material and physical sciences. Analyzing the NMR spectra can provide number of information about structure of compounds, interaction between molecules, reaction kinetics and data about conformation of macromolecules. Therefore, we took advantage the potential of NMR measurement for studies of broadly used biopolymers on both molecular and macroscale level.
Originally, we applied NMR measurement for quantitative assessment of polymerization rate of methacrylated biopolymers. We found NMR technique as the most reliable tool to establish optimal polymerization conditions for deeper understanding of photocuring process and reduce negative implication of UV light and photoinitator to living cells.
Furthermore, applying the NMR data we were able to establish enhancing printability technique of hydrogels based on methacrylated biopolymers by pre-crosslinking approach. For instance, methacrylated hyaluronic acid (HAMA) and methacrylated alginate (ALGMA) are widely used as additives to hydrogel-based bioinks but as single-component hydrogel suffer from numerous drawbacks. Utilization of pre-crosslinking of low-percentage methacrylate solutions allows to improve their printability. The application of the presented method of preparing biomaterials solutions provides new possibilities for precise extrusion-based 3D printing. Moreover, restricted diffusion Nuclear Magnetic Resonance is a useful tool for a structure investigation of porous materials. However, a single measurement is relatively long. In order to accelerate it, the Time-Resolved method was applied. By this technique, the required time for the measurement is depleted from around one day to even less than an hour. This method was used in our research for studying the properties of Gelatin Methacryloyl (GELMA) hydrogel. Learning the diffusion coefficient (D) dependence on diffusion time (Δ) allows to calculate size of pores in the material and its tortuosity.

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THE INFLUENCE OF DIVALENT IONS PRESENT IN ZEOLITES ON THE SORPTION AND RELEASE OF ACTIVE SUBSTANCES

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Introduction

Zeolites are microporous materials that belong to the group of hydrated aluminosilicates. They are composed of a three-dimensional framework formed by $[AlO_4]^{5-}$ and $[SiO_4]^{4-}$ tetrahedra, which are connected through oxygen atoms. If all the tetrahedra were made up of silicon atoms, the framework would be electrically neutral. However, when a silicon atom is replaced by an aluminum atom, this substitution creates a charge imbalance, resulting in a negatively charged framework. To counterbalance this negative charge, positively charged ions are required. In natural zeolites, these ions are typically monovalent or divalent metal ions, such as sodium, calcium, potassium, or magnesium [1,2]. The charge neutralization by these ions is made possible by the zeolite's ordered structure, which features regularly arranged pores and cavities that facilitate the movement of ions to sites of negative charge [2]. The pore size in the zeolite structure typically ranges from 4 to 12 Å. These unique structural properties make zeolites valuable in a wide range of applications. In biotechnology and medicine, zeolites are employed for biomolecule separation, biosensor construction, tissue engineering (mainly as implants coatings to promote osteointegration), and gene therapy. Additionally, they can serve as systems for controlled drug delivery [1].
Due to the fact that various ions (calcium, magnesium, zinc) can be introduced into the zeolite structure, in this work, it was decided to check the effect of the cation type on the sorption and release of active substances. Substances with antibacterial activity and substances that improve osseointegration were selected [3-5].

Materials and Methods

The ion exchange process was performed by mixing 100 ml of a 0.5 M solution of the respective chloride salts ($CaCl_2$, $MgCl_2$, $ZnCl_2$) with 5 g of Na-13X zeolite. The mixture was stirred for 24 hours, followed by centrifugation. This procedure was repeated three times. Afterward, the material was washed three times with distilled water and dried in an oven for 24 hours at 100°C. The resulting materials after ion exchange were designated as Ca-13X, Mg-13X, and Zn-13X.
In the next step, the zeolites were placed in solutions of active substances: risedronate or epigallocatechin gallate, or ciprofloxacin. The amount of drug retained was determined using UV-Vis spectroscopy.
After the sorption step, a drug release step was performed using a simulated body fluid as the medium.
Zeolites both before and after sorption were characterized using SEM/EDS analysis.

Results and Discussion

The first key finding from the research was that the sodium form of zeolite (Na-13X) was not capable of sorbing the active substances. In contrast, zeolites containing divalent ions showed different behavior. Their ability to retain drugs suggests that the drugs coordinate on the surface of the zeolite. For risedronate, the highest retention occurred with the Zn-13X carrier, while the lowest was observed with the Ca-13X carrier. For epigallocatechin gallate, the greatest retention was also found with the Zn-13X carrier, whereas the least retention occurred with the Mg-13X carrier. In the case of ciprofloxacin, the Mg-13X carrier retained the most drug, while the Ca-13X carrier retained the least. These findings suggest that both the type of ion and the structure of the drug significantly influence sorption. No single cation consistently resulted in the highest degree of sorption across all drugs.
Importantly, the results obtained using SEM analysis indicate that no new crystallites precipitated on the surface of zeolites. This indirectly confirms the assumption that active substances are retained in the form of a monolayer. Additionally, EDS analysis confirms that drugs are distributed evenly on the surface of the materials.
An important factor in the development of drug carriers is the manner and duration of drug release. The studies demonstrated that there was no initial burst release; instead, the drug release was slow and controlled. For risedronate, the release was monitored over 100 hours, with 22% released from Ca-13X, 11% from Mg-13X, and 5% from Zn-13X. The release continued beyond this period, with results indicating that the process would be longest for Zn-13X and shortest for Ca-13X.
The release of epigallocatechin gallate was carried out over 18 days, showing that the extent of desorption varied depending on the cation present in the zeolite. By the end of the process, significantly more epigallocatechin gallate was released from the zinc zeolite compared to the other materials, which correlates with the higher amount of drug initially retained on the carrier.
For ciprofloxacin, the release behavior differed among the zeolites. With Ca-13X and Zn-13X, a larger amount of ciprofloxacin was released in the first hour, followed by a slower release in subsequent hours. In contrast, with Mg-13X, 25 µg of ciprofloxacin was released in the first hour, with an additional 25 µg released over the next 15 hours.

Conclusions

The presented results confirm that zeolites with divalent ions can be effectively used in the delivery of risedronate, epigallocatechin gallate and ciprofloxacin. The drugs are retained on their surface by coordination, thanks to which they are released in a controlled slow manner. Such materials can be used in implantology as an element of scaffolds or coatings of orthopedic implants.

Acknowledgements

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CALCIUM CARBONATE PARTICLES LOADED WITH SODIUM ALENDRONATE IMMOBILIZED IN BIOACTIVE LAYER ON POROUS CERAMIC SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

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Introduction

The increasing number of musculoskeletal disorders motivates tissue engineering specialists to design innovative solutions. Tissue scaffolds seem to be a promising approach to bone reconstruction and regeneration. The porous architecture provides tissue ingrowth and ensures support for rebuilding bone. Biomaterials for tissue engineering applications must have several features to interact with living tissue [1]. Among the biomaterials available with unique properties for use in bone tissue regeneration and reconstruction are ceramic biomaterials, such as zirconium oxide (ZrO_2). It is characterized by good stability and Young's modulus similar to the mineral phase of natural bone, therefore it may provide structural support for newly formed bone tissue [2]. Unfortunately, it belongs to the group of inert ceramics, which means that the interaction at the implant-tissue interface is limited [2]; however, the appropriate surface modification may provide the inert biomaterial with bioactive properties, which stimulates bone tissue regeneration [3]. Furthermore, the presence of biologically active substance in the place of action may also be beneficial as compared to traditional therapies. Sodium alendronate has been reported to inhibit osteoclast activity and bone resorption [4].

The aim of the study was to functionalize the surface of porous ZrO_2 scaffolds with a bioactive calcium-phosphate (CaP) layer to improve its bioactivity and to immobilize calcium carbonate ($CaCO_3$) microparticles loaded with sodium alendronate in the CaP coating.

Materials and Methods

Calcium carbonate particles (MPs) loaded with sodium alendronate (10% *wt*) were obtained with the use of the precipitation method. SEM was used to observe the size and shape of the particles, while ATR-FTIR was used to confirm the effectiveness of the encapsulation process. Moreover, the encapsulation efficiency (EE) and drug loading (DL) were quantitatively checked with the use of the OPA assay. The ZrO_2 scaffolds were coated with a CaP layer during the co-precipitation process in the concentrated simulated body fluid (SBF). MPs were immobilized in the CaP coating during this process by adding them to SBF. Scaffolds after each step of functionalization were observed with SEM and the drug release profile was assessed. Furthermore, a biological evaluation with the use of osteoblast-like MG-63 cells was carried out to investigate the cytocompatibility of manufactured scaffolds.

Results and Discussion

The obtained ceramic MPs were characterized by a spherical shape and size of around 1 μm. The use of ATR-FTIR allowed us to confirm the efficiency of the encapsulation process. Peaks of both $CaCO_3$ and sodium alendronate were visible for loaded MPs. The OPA assay showed that EE was equal to 37%, while DL reached almost 10%. Through the SEM observations, differences in scaffold microstructure between steps of the functionalization process were noticed. The CaP layer after the second step of the coating process was thicker, and more hydroxyapatite-like crystals were present on the scaffold surface. Moreover, the presence of MPs in the solution caused even more efficient precipitation of CaP crystals. Moreover, they were visible on the particles surface. The drug release tests showed that the burst release of the drug was observed for both particles themselves and those immobilized in the CaP coating. Total sodium alendronate release after 14 days was slightly lower for scaffolds compared to particles, but no statistically significant differences were observed.

To assess the cellular response, MG-63 cells were seeded on the scaffolds (100 000 cells/scaffold) and on the tissue culture polystyrene (TCPS) as a control and the culture was carried out for 7 days. After days 1, 3 and 7 the AlamarBlue assay and live/dead staining were performed to assess metabolic activity, proliferation and morphology of the cells. On day 1, scaffold with empty particles showed the best performance, while other samples exhibited no statistical differences compared to TCPS. Statistically the same results, compared to the control, were observed for all tested samples on day 3 of the culture. On day 7 cell proliferation on the scaffolds with empty particles was limited and the cell viability did not increase significantly for these samples compared to day 3. On the other hand, better results were obtained on day 7 for the rest of the investigated samples. Although statistical differences compared to control were observed, the cells were proliferating well. According to the results, all evaluated samples were non-toxic for the cell culture used in the study.

Conclusions

The presented method of manufacturing of $CaCO_3$ particles loaded with sodium alendronate allows one to obtain round particles characterized by relatively high encapsulation efficiency and sodium alendronate loading. MPs immobilization in the calcium-phosphate layer during the coating process increased the amount of CaP crystals that are being created on the surface. Scaffolds functionalized with the CaP layer and calcium carbonate particles loaded with sodium alendronate are cytocompatibile with MG-63 osteoblast-like cells. Presented scaffolds appear to meet the expectations for materials for bone tissue engineering and regeneration.

Acknowledgements

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PHAGOCYTOSIS OF AZITHROMYCIN LOADED MICROPARTICLES BY RAW264.7 MACROPHAGES

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Introduction

Macrophages play an important role in immune recognition, initiation, and regulation of the inflammatory response toward invading microorganisms and foreign particles, and their subsequent removal by phagocytosis. Microparticles (MPs) delivered to the lungs have to avoid phagocytosis before the drug is released. The features of the MPs that prevent phagocytosis are: elongated shape, neutral surface charge, and hydrophilicity [1], [2].
Herein, we manufactured and characterized parameters that influence on phagocytosis of inhalable polyanhydride MPs loaded with azithromycin (AZM). The phagocytosis of MPs by RAW264.7 macrophages was determined.

Materials and Methods

MPs were manufactured using oil-in-water emulsification with solvent evaporation. The copolymers of poly(sebacic acid) (PSA) and poly(ethylene glycol) (PEG) with molecular weights 250 kDa and 600 kDa (PSAEG250 and PSAEG600, respectively) were dissolved in dichloromethane (DCM). 2 ml of dissolved polymer (2% wt) was added to 12 mg of AZM and homogenised with ultrasonic probe (1 min, 40% amp.). The polymer with AZM was poured to 30 ml of 8% poly(vinyl alcohol) (PVA) aqueous solution. The mixture was subjected to magnetic stirring (15,000 rpm) for 2 h until DCM evaporation. After MPs formation PVA was removed by repeated rinsing in MilliQ water, freezing, and lyophilization. The successful preparation of MPs was confirmed by scanning electron microscopy (SEM). Additionally, the Zeta potential of MPs was measured. Wettability of the materials processed in a form of foils was measured by sessile drop method.
RAW264.7 macrophages (European Collection of Cell Cultures) were seeded in a 96-well plate at 100,000 cells/ml (10,000 per well) and incubated in a 5% CO_2 humidified atmosphere. After 24 h, the medium was removed and MPs (stained with Nile Red fluorescent dye) suspension was added to the wells concentration of 50 µg/ml, which was found to be safe, i.e. not affecting RAW264.7 cells viability. After 2, 4, 6, and 24 hours, the cells were fixed and the fluorescence of the MPs was measured. Phalloidin/DAPI staining was also performed.

Results and Discussion

MPs were successfully manufactured using the emulsification method. Depending on the used polymer the MPs had different morphologies: PSAEG250_AZM were round while PSAEG600_AZM were both round and elongated (FIG. 1). The Zeta potential was similar for both MPs (4.7 ± 0.5 and 4.4 ± 0.6 for PSAEG250_AZM and PSAEG600_AZM, respectively).

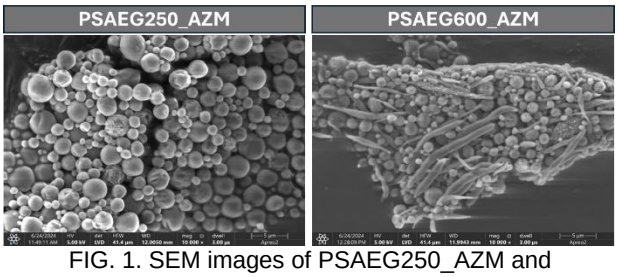

FIG. 1. SEM images of PSAEG250_AZM and PSAEG600_AZM MPs.

The phagocytosis of MPs was determined based on their fluorescence signal. Despite the elongated shape, the PSAEG600_AZM MPs were uptaken faster than the PSAEG250_AZM MPs (FIG. 2). This behaviour could be connected with the hydrophilicity of the copolymers. The wettability test showed that PSAEG250 was more hydrophilic than PSAEG600 (56º ± 3.0º and 71.0º ± 8.4º, respectively).

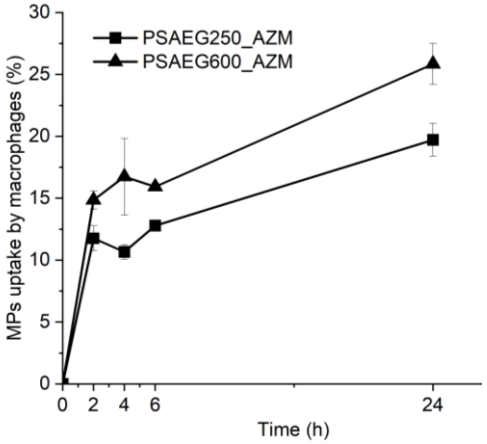

FIG. 2. MPs uptake by RAW264.7 macrophages.

Conclusions

The results show that PSAEG600_AZM MPs were uptaken faster by macrophages than PSAEG250_AZM MPs. However, more experiments are necessary such as co-cultures of macrophages with bacteria to assess if MPs have the ability to kill bacteria before being uptaken by macrophages.

Acknowledgements

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CIPROFLOXACIN AS ANTIBACTERIAL AGENT IN DENTAL COMPOSITES

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Introduction

Ciprofloxacin (CF) is a well-established fluoroquinolone antibiotic known for its efficacy against both gram-positive and gram-negative bacteria, with minimal side effects [1]. Studies have demonstrated that CF is particularly potent against oral bacteria, including the highly cariogenic *Streptococcus mutans*, and it was found to be the most effective antibiotic among those tested [2]. CF has been successfully incorporated into glass-ionomer cement (GIC), resulting in reduced cytotoxicity and enhanced antibacterial properties [3]. Additionally, in resin-based composites (RBC), CF has been used in the form of a dimethacrylate oligomer, which releases the antibiotic as the resin undergoes hydrolytic degradation [4].

In these studies, the action of CF was combined with the beneficial effects of another factor, which is magnesium. Previous research has documented the successful use of MgO particles as antibacterial components in glass-ionomer cement [5] and resin-based composites [6], demonstrating effectiveness against cariogenic microorganisms. Additionally, Mg-doped ZnO nanoparticles added to dental resin help buffer the acidic environment, thereby preventing biofilm formation [7]. The role of Mg^{2+} in oral health is even more significant, as magnesium ions can integrate into the enamel structure, enhancing its hardness and resistance to cracks by promoting the formation of smaller hydroxyapatite crystals during the remodeling process [8].

In this research CF and Mg were incorporated into the pores of a zeolite – a perspective RBC filler. Such filler was used together with methacrylic resins and a polymerization initiating system to obtain a composite with potential dental applications. Its basic physicochemical properties, as well as antibacterial activity and magnesium release, were examined.

Materials and Methods

First, a magnesium zeolite was prepared by exchanging sodium ions with magnesium, and then ciprofloxacin was adsorbed onto its surface according to the methodology described in [9]. The resulting fillers, both magnesium (Mg-X) and magnesium with CF (Mg-X-CF), were mixed with the methacrylate matrix (Bis-GMA and TEGDMA) of the composite to achieve a 65% volume content. The mixture was combined with photopolymerization initiators (CQ and EDMAB), shaped in PTFE molds, and cured using a dental LED lamp.

The fillers were characterized using a scanning electron microscope (SEM), X-ray diffractometer (XRD), nitrogen adsorption analyzer, FTIR microscope, and thermogravimetric analyzer (TGA).

The following properties of the obtained composites were examined: degree of conversion (DC), depth of cure (DOC), compressive strength (CS), flexural strength (FS), sorption (SP), and solubility (SL). Additionally, the magnesium release profiles were obtained, and the bactericidal properties were assessed.

The obtained results were subjected to statistical analysis with a significance level of $\alpha=0.05$.

Results and Discussion

The obtained results demonstrated the efficacy of ion exchange and ciprofloxacin retention on the surface of the starting sodium zeolite.

The composites produced based on them exhibit a high degree of conversion (over 65%), significant curing depth (over 1.5 mm), high compressive strength (over 200 MPa), and bending strength (over 50 MPa, classifying them as materials with potential use in filling non-load-bearing tooth surfaces). They also show moderate sorption (over 50 μg/mm³) and satisfactory solubility (below 7.5 μg/mm³). Additionally, they demonstrate the ability to release magnesium ions in both aqueous and acidic cariogenic environments for at least 28 days. Furthermore, both materials are effective against *Escherichia coli*, achieving over 30% reduction compared to control, and the composite containing ciprofloxacin also combats *Staphylococcus aureus* with over 80% reduction compared to control.

Conclusions

In summary, attractive fillers have been obtained and used in composites with dental potential, which, in addition to satisfactory basic physicochemical properties, also exhibit antibacterial activity. The addition of ciprofloxacin provides the tested composites with enhanced antibacterial effects.

Materials of this type, due to their antibacterial activity, can be used temporarily even if their other properties do not meet expectations for permanent dental restoration.

Acknowledgements

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CHITOSAN AND CHITOSAN/ POLY(VINYL ALCOHOL) MICROCAPSULES AS CARRIERS OF GALLIC ACID

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Introduction

Hydrogels are one of the most versatile drug carriers. It is usually pretty ease to encapsulate a drug within hydrogel capsule, and at the same time they allow stability and controlled release. Their main characteristic, i.e. high water content, resembles biological tissues composition, often leading to improved biocompatibility and reduced toxicity. They can be also designed and modified to respond to specific stimuli (e.g. pH or temperature), hence creating possibility for targeted or sustained delivery [1]. Both natural and synthetic polymers can be used to create such hydrogel carriers. Biopolymers (e.g. chitosan, alginates) are chosen for their biocompatibility, biodegradability, and bioactivity. However, lack of mechanical strength and issues with reproducibility are their important drawbacks. In turn, synthetic polymers (e.g. poly(vinyl alcohol)) provide better structural integrity with defined and adjustable properties but they may rise compatibility issues. Blending these two is a chance to combine the advantages and limit shortcomings. In this study microcapsules based on chitosan (CS) and a blend of CS and poly(vinyl alcohol) (PVA) were designed to encapsulate gallic acid (GA). This naturally occurring polyphenol is known for its antioxidant, anti-inflammatory, and possible antimicrobial properties. Hence, it can be used in many biomedical applications as a bioactive compound.

Materials and Methods

First basic polymer solutions were prepared. Chitosan (Sigma-Aldrich, high Mw) was dissolved in 1% acidic acid to reach 1.5% concentration, while PVA was dissolved in distilled water under heating (70°C) with a final concentration of 10%. Both solutions were left on a magnetic stirrer for 24 hours. CS/PVA blend was created by mixing CS and PVA solutions in 9:1 ratio. GA-modified systems were obtained by adding 20wt.% of gallic acid powder to the polymer solutions. Finally, CS, CS/PVA, CS+GA, and CS/PVA+GA microcapsules were fabricated by dropping (0.7 mm needle placed 6 cm above) appropriate solution to a constantly stirred gelling bath (5% sodium tripolyphosphate (TPP), 1% vanillin, 1% Span80) with a volume ratio of polymer solution to the gelling solution set at 1:10. The formed microcapsules were crosslinked for 48 h, subsequently centrifuged, washed with isopropanol, ethanol, and distilled water, and lastly dried in a laboratory drier (24h, 50°C).
Microstructure (digital microscope), chemical stability (weight loss, water absorption), and structure (FTIR-ATR) were evaluated for each sample. Gallic acid release was measured with a UV-VIS method. 5 mg of each sample was placed in a tube filled with 5 ml PBS and incubated at 37°C. At defined time points, 1 ml of medium was collected and replaced with a fresh one. Absorbance at 260 nm was measured to define a release profile.

Results and Discussion

The obtained CS and CS/PVA microcapsules were round and homogenous with an average diameter of {1411 µm; 621 µm} and {1286 µm; 565 µm} in a wet and dry state, respectively (FIG. 1).

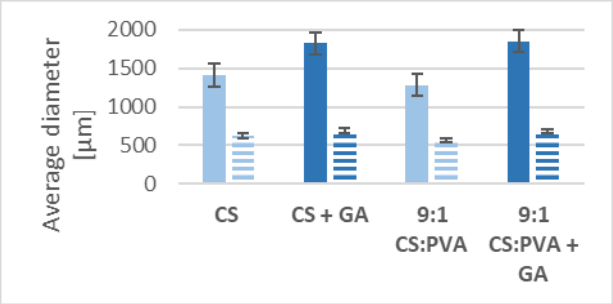

FIG. 1. Average diameter value for wet and dry (striped) microcapsules for each material (n=40).

Addition of gallic acid resulted in a change of colour and size increase. Modified microcapsules were almost black and visibly bigger when hydrated, although similar in size to the unmodified equivalent after drying (FIG. 2). Higher water absorption for GA-containing samples was also confirmed through quantitative analysis.

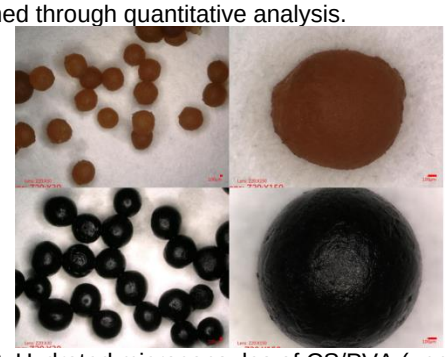

FIG. 2. Hydrated microcapsules of CS/PVA (upper) and CS/PVA+GA (lower); scale bar: 100 µm.

FIG. 3 shows cumulative release of the bioactive compound. Probably due to the higher water absorption, GA was released faster from the CS/PVA system. However, both release profiles were similar with no evident initial burst release

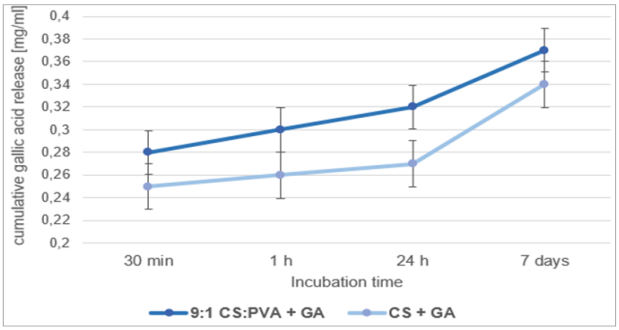

FIG. 3. Cumulative gallic acid release from CS+GA and CS/PVA+GA samples

Conclusions

Both chitosan and chitosan(poly(vinyl alcohol) blends can be used as carriers of gallic acid. Detailed studies are needed for their proper characterization.

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HYDROGEL DRESSINGS ENRICHED WITH QUERCETIN LIPID CARRIERS FOR THE TREATMENT OF CHRONIC WOUNDS

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Introduction

Skin wounds are a serious global medical problem that affects approximately 5% of people in developed countries, of which 40% experience complications in the healing process [1]. Chronic wounds are characterized by high levels of reactive oxygen species (ROS), which causes oxidative stress and leads to inflammation. Low levels of ROS are beneficial in protecting tissues from infection, stimulating fibroblast proliferation and angiogenesis. Therefore, regulation of the redox balance through the use of antioxidants that keep ROS levels low is a target for new therapies [2]. In our work we intend to develop a gellan gum (GG) – sodium alginate (ALG) based wound dressing enriched with lauric acid (LAU) carriers for controlled delivery of quercetin (QE) – which has antioxidant properties for the treatment of chronic wounds.

Materials and Methods

The lipid particles were manufactured from LAU by oil-in-water (o/w) hot emulsification. The oil phase was LAU with addition of 5% QE prepared at 65°C for 30 min and homogenized using an ultrasonic probe for 90 s at 40% amplitude. Oil phase was poured into water phase, which was a 10% solution of poly(vinyl alcohol) (PVA) at 65°C and homogenized for 3 min at 40% amplitude. In order to create particles, prepared emulsion was poured into liquid nitrogen. After thawing particles were centrifuged using 15,000 rpm for 30 min, washed three times with MilliQ–water, freeze dried for 48 h and stored at -20°C for further use. The size and polydispersity index (PDI) were characterized by dynamic light scattering (DLS). Quercetin release studies from lipid carriers were conducted using Franz diffusion cells. The release medium was a mixture of HPLC-water and absolute alcohol (65:35% *v/v*). The experiment was carried out at 37°C using stirring speed of 100 rpm. After 2, 4, 6, 24, 48 and 72 h, 0.5 ml of receptor medium was withdrawn and replaced with fresh medium. Quercetin concentration in samples was measured using the HPLC method (n = 3). *In vitro* human skin permeation studies were performed using Franz diffusion cells at 37°C and a stirring speed of 100 rpm. First skin was thawed for 30 min and hydrated in PBS solution at 37°C for another 30 min. Then the skin was cut and mounted on the Franz diffusion cell, with the dermis facing the receptor compartment. Particles in PBS solution were added to the donor compartment. Samples from receptor compartment were collected after 24 h. In order to extract quercetin from skin, the particles were removed from the donor compartment. The surface of the skin was washed with PBS solution. Epidermis and dermis were separated, weighted and transferred to different tubes with 1 ml of ethanol. Then samples were homogenized 6 x 90 s (3,000 rpm), centrifuged for 5 min (10,000 rpm) and filtrated prior to HPLC analysis. Hydrogel matrices were prepared by dissolving GG and ALG in a ratio of 10:1 in MilliQ-water, then equal volumes of aqueous suspension of QE particles and cross-linking agent ($CaCl_2$) were added. Concentrations of components in the final mixture were 2% *w/v* GG/ALG, 0.1% *w/v* particles and 0.06% *w/v* $CaCl_2$, which was then cast into a Petri dish and cooled down to 4°C. After cross-linking round samples were cut (12 mm in diameter and 4 mm in height and freeze dried for 48 h. Samples were characterized by optical and scanning electron microscopy (SEM). *In vitro* cytotoxicity studies were performed in contact with L929 fibroblasts. Swelling capacity and mass changes were tested as a function of incubating in PBS solution.

Results and Discussion

Quercetin loaded lipid particles with a size of 984 ± 189 nm and PDI of 0.219 were successfully manufactured. Encapsulation efficiency of QE was about 63%, and drug loading was 3%. The release profile of QE is characterized by an initial burst, which was observed for all tested samples. In the following hours, the level of the released substance continues to increase, but it is released more slowly and in smaller amounts. During the skin permeation studies on human skin after 24 h, the presence of quercetin in both epidermis and dermis was confirmed. About 40% of the released QE was found in the epidermis and about 8% of the released QE was found in the dermis. After the freeze-drying process of GG/ALG samples enriched with QE particles, highly porous materials with homogeneously deposited particles on their surface were obtained. The swelling capacity of the dressing prototypes was 2680 ± 320% with the 91% of remaining mass. Materials were not found to be cytotoxic for the L929 fibroblasts.

Conclusions

In conclusion, we successfully manufactured lipid particles loaded with QE with prolonged release and proved the presence of the released QE in different layers of the skin. Next, we manufactured GG/ALG matrices enriched with particles and characterized them. *In vitro* studies showed that manufactured materials are not cytotoxic for fibroblasts, which allows for further *in vitro* studies on other cells involved in the healing process. Following studies will also include determination of QE release profile from GG/ALG matrices.

Acknowledgements

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OPTIMISATION OF PLGA MICROPARTICLES MANUFACTURING USING A RAYDROP MIRCOFLUIDIC DEVICE

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In biomedical sciences, it is essential to develop degradable polymer microparticles with controlled size and properties. These particles can be used as drug carriers or scaffolds in bottom-up approach to tissue engineering [1-3].
The purpose of the study is to optimise the production of homogeneous spherical poly(L-lactide-*co*-glycolide) (PLGA) microparticles of specific size and low polydispersity using microfluidic device and to determine the impact of flow parameters on particle sizes, including the flow rate and the flow rate ratio between water and oil phases.

Materials and Methods

The water phase (WP) was prepared at a concentration of 0.5% (w/v) as aqueous solutions of poly(vinyl alcohol) (PVA, Mowiol 4-88). As oil phase (OP), PLGA (85:15, Mn =100, d = 2.0) was dissolved in dichloromethane (DCM) at a concentration of 2% (w/v). Microparticles were obtained in a microfluidic device (RayDrop, Fluigent; microchip picture is shown in FIG. 1) The flow rates of the WP were 25, 50, 100, 200, 400 µl/min and the flow rates of the OP were 10, 20, 40 µl/min. The morphology of the microparticles was evaluated using an optical microscope (Axiovert, Zeiss), and their size was measured with ImageJ software based on the number of 100 adjacent microparticles.

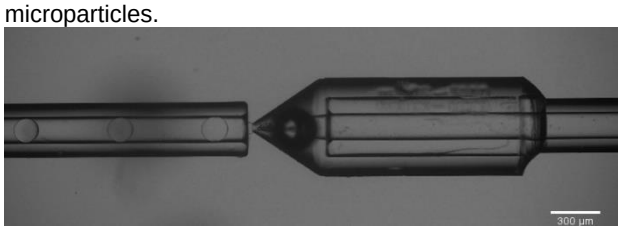

FIG. 1. Generating PLGA droplets in RayDrop microchip.

Results and Discussion

The results indicate that microparticles produced using a microfluidic device exhibit a spherical shape with a low coefficient of variation. The sizes of these microparticles range from 22.9±1.9 µm (FIG. 2A) for WP flow rate 400 µl/min and OP 10 µl/min to 72.9±32.6 µm (FIG. 2B) for WP flow rate 25 µl/min and OP 10 µl/min (TABLE 1). The size of the particles is influenced not only by the phase flow rate ratio but also by the actual flow velocities. As the phase flow rate increased while maintaining the same OP ratio, the particle size also increased. To obtain a large number of particles in a short time, it is necessary to set the WP flow rate to 400 µl/min and vary the OP flow rate to adjust the size. However, to maintain particle homogeneity, it is better to vary the WP flow rate and set the flow rate of OP to 10 µl/min. The most optimal OP:WP ratios for generating uniform particle sizes are 1:5, 1:10, 1:20, and 1:40. When the OP:WP ratio was 1:2.5, it became challenging to maintain a steady flow, resulting in high polydispersity of the particles. Furthermore, at ratios of 1:1.25 or lower, it was impossible to produce particles.

TABLE 1. Size of PLGA microparticles (in µm). X - indicates the flow rates at which microparticles could not be obtained.

		Flow rate of OP [µl/min]		
		10	**20**	**40**
Flow rate of WP [µl/min]	**25**	72.9±32.6	X	X
	50	35.7±2.8	48.7±7.6	X
	100	33.7±2.7	43.7±5.6	68.9±17.6
	200	26.4±2.1	40.1±4.8	58.3±11.7
	400	22.9±1.9	36.3±4.2	47.1±8.9

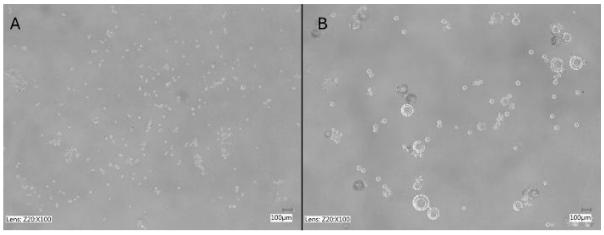

FIG. 2. PLGA microparticles obtained with flow rates: A - WP 400 µl/min, OP 10 µl/min, B - WP 25 µl/min, OP 10 µl/min.

Conclusions

The study shows that microfluidic technology allows to obtain PLGA microparticles with low polydispersity and defined size, which can be controlled by the velocity of both water and oil phases and their ratio.

Acknowledgements

This study was supported by the subsidy (No 16.16.160.557) and by the Program “Excellence Initiative – Research University” for the AGH University of Krakow.

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THE INFLUENCE OF CHEMICAL TREATMENT ON THE PHYSICOCHEMICAL AND MECHANICAL PROPERTIES OF TITANIUM 3D PRINTS

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Introduction
Additive manufacturing of components and parts from various materials has become popular in various branches of life, including medicine. Many metallic implants, including those requiring high porosity, are already being manufactured in this way. The printing process, which involves laser sintering of powders, leaves many weakly bound particles on the surface of the print. Their presence is fraught with the risk of uncontrolled release into the body after the implantation process and requires the use of additional treatments leading to the cleaning of the print. The most commonly used is chemical etching, which, in addition to cleaning the print, also leads to structural and geometric changes in the print. This, consequently, can affect the reduction of their mechanical strength. The purpose of this study was to determine the relationship between the chemical treatment parameters of openwork 3D prints made of Ti6Al4V alloy and their change in physicochemical and strength properties, with particular emphasis on compressive strength.

Materials and Methods
Samples in the form of openwork cylinders with a height of 9.5 mm and a diameter of 7 mm were used for the study. They were fabricated by 3D printing (SLM) technique from Ti76Al4V titanium alloy powder. The samples were subjected to plasma etching (argon and oxygen plasma), chemical etching (HF acid solution) and chemical and plasma etching. The effect of the processes was evaluated by mass measurements, scanning electron microscopy, computed microtomography, EDS analysis and compressive strength measurements.

Results and Discussion
Rozpatrując wyniki otrzymane w wyniku ważenia próbek Considering the results obtained by weighing the samples before and after the etching processes to which the test samples were subjected, it can be seen that the chemical etching process significantly reduces the weight of the samples, which is associated with a loss of material and a decrease in compressive strength. However, the loss of material at the level of several percent was lower than literature reports, where it was shown that weight losses reached even more than 30%. The plasma etching process, on the other hand, led to an increase in the weight of the samples tested, which was due to the oxidation process of the samples during plasma etching, the fused powder was not fully removed. Samples subjected only to plasma etching had the most similar mass to control samples.
Optimal in terms of removing unmelted powder particles, was the use of chemical etching or two-step etching (chemical followed by plasma). After using these methods, there are almost no free powder particles left. In both cases, the undesirable effects of digesting the material, deteriorating the mechanical strength of the structure, were also not observed.
Regardless of which etching process the sample was subjected to, all compression strength graphs showed a maximum, after which brittle fracture of the samples and complete failure occurred. The average value of the maximum compressive force had the highest value sequentially for the control samples (7.58 kN), followed by the samples subjected to plasma etching (7.4 kN), the samples subjected to chemical etching (6.86 kN), and the lowest value was exhibited by the samples subjected to both chemical and plasma etching processes (6.3 kN). Considering the high porosity (more than 50%), these mechanical properties are high enough not to disqualify, either method from possible application.

Conclusions
- The use of plasma etching did not fully clean the surface of the prints.
- Chemical etching with a 2% HF acid solution effectively removed unmelted Ti6Al4V powder particles from the implant.
- Optimal in terms of removal of unmelted powder particles and at the same time the least possible loss of scaffold wall material is the use of two-step etching (chemical followed by plasma).
-The etching processes of the samples affected the compressive strength. There was a reduction in compressive strength in each of the three groups of etched samples.

Acknowledgements
Work funded under the 2nd Priority of the European Funds for Modern Economy 2021-2027 (FENG) Program, Grant Agreement No. FENG.02.07-IP.05-0370/23, Project Title: Purification and bioactivation of titanium 3D prints for biomedical applications.

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THE EFFECT OF RESVERATROL AND STRONTIUM-DOPED BIOACTIVE GLASSES ON CHITOSAN-BASED HYDROGELS

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Introduction

Contemporary biomedical engineering is continually seeking new, innovative materials that can be utilized in regenerative medicine and tissue engineering. Among the promising areas of research are hydrogels, particularly those based on biopolymers such as chitosan, due to their biocompatibility, biodegradability, and ability to form three-dimensional structures [1]. The combination of chitosan with bioactive glass, which supports the regeneration of bone and cartilage tissue through its osteoconductive and osteoinductive properties, represents an intriguing strategy in the design of advanced biomaterials [2,3]. Additionally, enhancing such materials with highly bioactive substances, like resveratrol, can significantly increase their therapeutic potential, thanks to their antioxidant properties and ability to support the regeneration of human tissues [4].

Materials and Methods

The aim of the research was to develop and produce hydrogel materials based on chitosan and whey protein isolate, crosslinked with functionalized dextran, and enriched with high-calcium bioactive glass and resveratrol. The specific focus of the research was on injectable and lyophilized chitosan-based hydrogels enriched with resveratrol at concentrations of 2%, 5% and 10% and bioactive high-calcium glass doped with strontium A2Sr5, with the following composition: 40 mol% SiO_2, 49 mol% CaO, 6 mol% P_2O_5 and 5 mol% SrO_2. The addition of biologically active substances was studied with regard to their impact on the physico-chemical, mechanical, rheological, antioxidant, and biological properties. The following tests were carried out: FTIR infrared spectroscopy, SEM scanning electron microscopy with additional EDS analysis (FIG. 1, FIG. 2), compressive strength testing, antioxidant properties, evaluation of resveratrol release, testing of rheological parameters, evaluation of self-healing and biological properties in vitro using RAW 264.7 murine macrophage cell line.

Results and Discussion

The results clearly indicate that bioactive glass has a significant effect on improving the mechanical parameters of the developed materials, as well as their bioactivity, mineralization, flexibility and structural stability, while resveratrol significantly improves the antioxidant and mechanical properties of hydrogels.

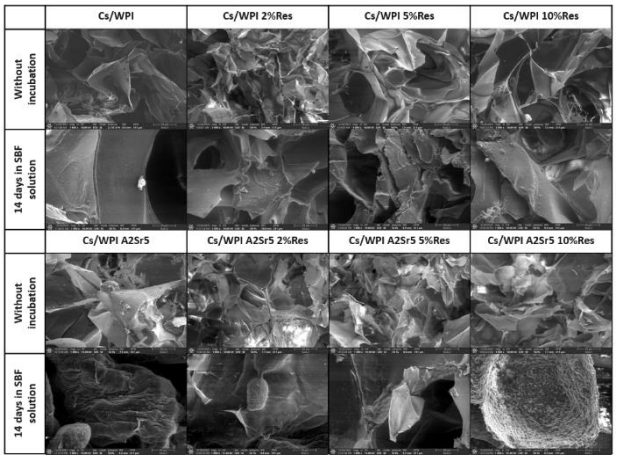

FIG. 1. SEM microscope images before and after 14 days of incubation materials in SBF solution.

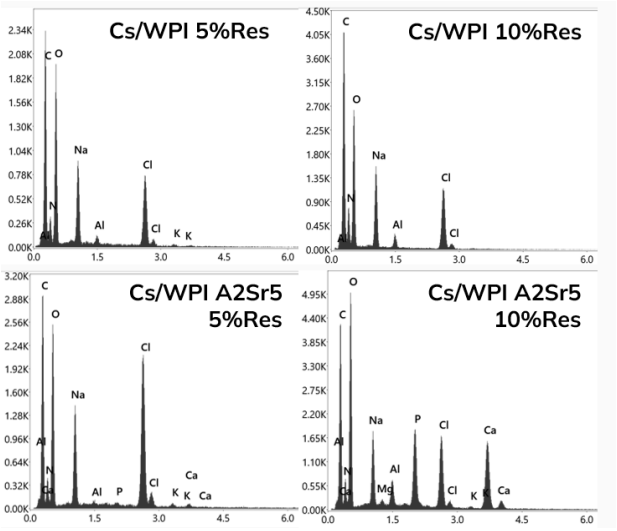

FIG. 2. EDS analyses of materials after 14 days of incubation in SBF solution.

Conclusions

The developed and obtained hydrogels have promising potential for use as innovative injectable materials and lyophilized scaffolds for tissue engineering for bone and cartilage tissue regeneration.

Acknowledgements

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HOW BIOLOGICALLY ACTIVE COMPOUNDS INFLUENCE THE PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF CHITOSAN HYDROGELS

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Introduction

Chitosan-based hydrogel materials are an intriguing class of biomaterials due to their advantageous properties, such as biodegradability, biocompatibility, high water content, great swelling and antibacterial activity. They are frequently used as wound dressings, drug delivery systems and tissue engineering materials. Their versatility, safety, and modifiability make them ideal candidates for therapeutic applications [1]. Chitosan hydrogels can be enhanced with biologically active substances such as bioactive glasses – which are valued for their ability to integrate with biological tissues, stimulate regenerative processes and reduce infection risks [2] or retinol – known for its anti-aging properties and skin conditioning benefits [3].

Materials and Methods

The focus of this study is on chitosan-based hydrogels enriched with bioactive glass doped with strontium A2Sr5 (40 mol% SiO_2, 49 mol% CaO, 6 mol% P_2O_5 and 5 mol% SrO_2) and retinol at concentrations of 2%, 5% and 10%, crosslinked with dextran dialdehyde. A key aspect is that these materials were produced in two forms: injectable hydrogels and lyophilized, highly porous scaffolds.
The aim of this research was to evaluate the impact of both biologically active substances on the physicochemical and biological properties of the developed materials. The materials were incubated in SBF solution to assess their mass loss, swelling and mineralization. Mechanical and rheological parameters (FIG. 1) were also tested. The study also investigated retinol release (FIG. 2) and self-healing properties. Moreover, biological studies were conducted on RAW 264.7 murine macrophage cells.

Results and Discussion

Based on the obtained results, it was found that the incorporation of A2Sr5 bioactive glass and retinol into chitosan-based hydrogels, produced in two forms, significantly influenced their structure and properties. The presence of bioactive glass improved the mineralization and bioactivity of the hydrogel scaffolds. Additionally, it reduced swelling and the mass loss of the materials during incubation in SBF solution. Both forms of hydrogels enriched with A2Sr5 bioactive glass achieved better results for the tested mechanical parameters. The introduction of both biologically active substances shortened the crosslinking time of injectable hydrogels, enhanced their elastic properties and positively affected the self-healing process. Furthermore, it was observed that the presence of A2Sr5 bioactive glass increased the metabolic activity and survival rate of RAW 264.7 murine macrophage cells.

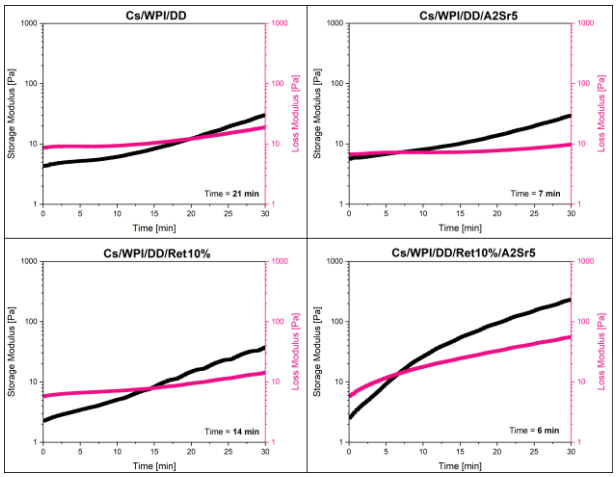

FIG. 1. Crosslinking time of obtained injectable chitosan hydrogels with A2Sr5 bioactive glass and retinol.

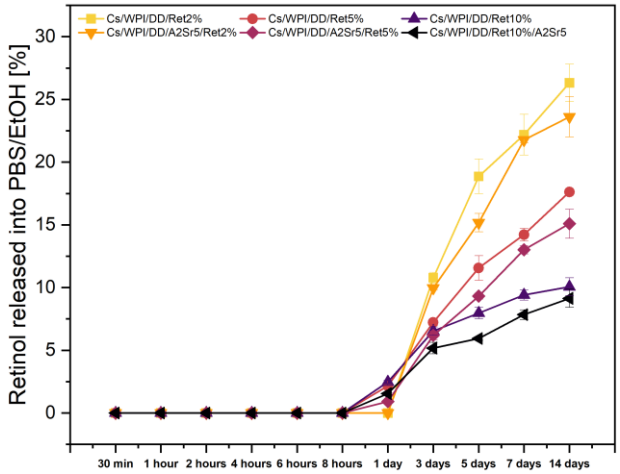

FIG. 2. Release of retinol during incubation of lyophilized chitosan hydrogel scaffolds with A2Sr5 bioactive glass and retinol into PBS/EtOH solution.

Conclusions

Enrichment of the chitosan matrix with strontium-doped bioactive glass and retinol led to the development of innovative materials in the form of injectable hydrogels and lyophilized, porous scaffolds. Both forms of the hydrogels have high potential for use as multifunctional biomaterials for tissue engineering for bone and cartilage regeneration.

Acknowledgements

This work was supported by the National Science Centre, Poland (Grants no. 2023/49/N/ST11/02999) and the "Excellence Initiative – Research University" program for AGH University of Krakow.

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THE INFLUENCE OF THE Ar AND O PLASMA POST-PROCESSING METHOD FOR CLEANING DMP-PRODUCED METALLIC 3D PRINTS ON THE PROCESS EFFICIENCY AND BIOLOGICAL RESPONSE

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Introduction

The 3D printing technology with the DMP (direct metal printing) method allows the production of metal elements with complex geometry. The DMP method facilitates the production of metal objects with high precision. One of the important applications of additive manufacturing is the production of medical implants. A significant challenge in the production of medical devices using the DMP technique is the so-called postprocessing. An inadequately conducted cleaning process after production can lead to the presence of metallic particles that were not bound during laser melting.
Commonly available post-processing methods are not effective in removing superficial powder debris in such implants and can therefore be hazardous to the patient. Electrochemical methods are very effective in removing unbound particles [1], but at the same time they cause significant volume and mass losses in prints [2], which affects their strength [1,3]. Comparing different methods of surface postprocessing treatment (chemical, electrochemical, mechanical, plasma), it was found that plasma treatment is most promising solution based on a literature review. The conducted research was aimed at verifying the effectiveness of the plasma purification process from unbound particles present on metallic 3D prints.

Materials and Methods

The samples were printed using the DMP method from titanium alloy powder (LaserForm® Ti Gr23 A) with a particle diameter of up to 40 μm using a ProX DMP 320 3D printer. The elements printed on its basis have a chemical composition consistent with the commonly known Ti-6Al-4V ELI alloy. The initial samples (3D printed) were divided into two groups: 1) samples without sandblasting after the manufacturing process, 2) samples sandblasted after manufacturing.
The plasma cleaning process of the prints was carried out using the plasma etching method in the RF PACVD method. Oxygen or argon was used independently as the working gas. During the process, constant values of the gas flow, pressure in the working chamber of the generator power and the negative polarization potential of the RF electrode were maintained. For both gases, three series of processes were carried out, differing in duration -1, 2 and 4 hours. Samples before and after plasma cleaning were observed using SEM and their composition was analyzed using EDS. To compare the surface topography of samples before and after the plasma cleaning process, a study was performed using a contact profilometer. The change in the mass of samples before and after cleaning, the biological response in vitro on osteoblast-like cells Saos-2 were also studied by analyzing cytotoxicity (MTT test) and proliferative capacity by culturing cells directly on surfaces and verifying their morphology and the area occupied on the sample by SEM observations.

Results and Discussion

SEM observation showed that the mechanical treatment caused a change in the surface geometry instead of removing metal powder particles from the sample surface. In the case of etching the unsandblasted samples in both oxygen and argon, the number of unbound particles on the surface was not reduced. The longer cleaning performed caused the loss of the regularity of the shape of individual particles. X-ray microanalysis of both types of samples after etching in both oxygen and argon showed that the applied plasma cleaning parameters did not significantly affect the chemical composition of the tested prints. Comparing the results for the series of sandblasted samples, a decrease in the values of all roughness parameters can be seen with the extension of the plasma cleaning time. The greatest difference is observed between the control samples and those subjected to plasma cleaning in an argon atmosphere for 2 hours. Saos-2 cell cultures showed that plasma etching activated the sample surfaces contributing to faster cell proliferation.

Conclusions

Plasma cleaning affects the properties and surface structure of printed Ti6Al4V samples, changing their roughness. For unsandblasted samples, plasma cleaning causes a change in the regularity of the shape of the particles, and the surface of sandblasted samples is smoothed. The plasma treatment process in both argon and oxygen does not significantly change the chemical composition of the sample. The surfaces of plasma-modified samples become more hydrophilic, which translates into a greater ability of cells to adhere to their surface. Purification carried out in argon allows for obtaining a surface with greater activity in relation to osteoblast-like cells of the Saos-2 line. Processes conducted in this gas for 2 and 4 hours give the effect of rapid cell colonization and their increased proliferation observable after 24 hours from the time of culturing. At the same time, plasma purification processes do not introduce changes that would result in toxicity of the analyzed samples - for all processes, cell viability measured by the XTT test remained at the level of >70%. In summary, plasma treatment is a good method of cleaning and modifying metal elements manufactured using 3D printing technology, allowing for the purification effect while improving their physicochemical and biological properties.

Acknowledgements

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OPTIMIZATION OF DRUG MICROCARRIER SIZE FOR TARGETED LUNG DELIVERY

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Introduction

The deposition site of inhaled microparticles acting as drug carriers in the respiratory tract is highly dependent on their size. Particles that are too small (<1 μm) can be easily expelled from the lungs during exhalation, while those that are too large (>5 μm) may not reach the infected areas of the lungs [1]. Additionally, bronchial narrowing caused by increased mucus secretion, particularly in patients with COPD, impairs airflow and prevents proper ventilation. Insufficient airflow generation during inhalation presents a challenge for inhalation therapy, as it reduces the ability to draw particles from the inhaler. To improve the usability of inhalation devices and enhance treatment effectiveness, the aerodynamic properties of drug carriers can be modified. Preventing the agglomeration of particles can enhance the aerodynamic properties, increasing the efficiency of the inhaled powder and ensuring even particle distribution in the lungs. Furthermore, the absence of large particle clusters makes inhalation far easier [2], [3]. In this study, various modifications were explored to assess their impact on the aggregation of poly(sebacic-*co*-ethylene glycol anhydride) with Mw = 250 kDa (PSAEG250) based microparticles loaded with model antibiotic azithromycin.

Materials and Methods

Microparticles (MPs) were produced using a solid-in-oil-in-water (S/O/W) emulsification method (FIG. 1). In this process, the solid phase, azithromycin (Az), was combined with an oil phase containing PSAEG250 dissolved in dichloromethane (DCM). Az, at a concentration of 30% relative to the polymer, was dispersed and encapsulated within the oil phase using ultrasound. The resulting mixture was then added dropwise to the water phase, which consisted of a poly(vinyl alcohol) (PVA) solution. MPs were formed by stirring all phases continuously for 2 h, followed by solvent evaporation. Then, the MPs were centrifuged and washed three times with Milli-Q water to eliminate surfactant residues, frozen with liquid nitrogen, and finally lyophilized.

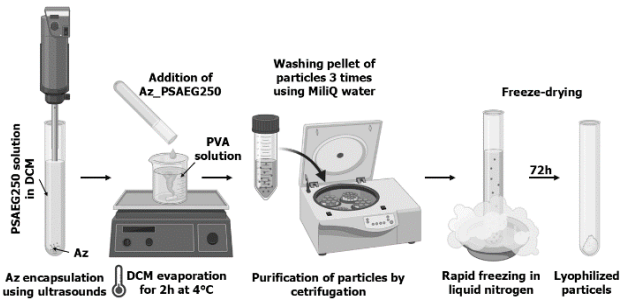

FIG. 1. The synthesis method for Az-loaded PSAEG250 microparticles, which was further modified. The image was created using BioRender.com

During the optimization process, various parameters were adjusted, and different anti-agglomeration agents were tested. The modified parameters included varying volume ratios of oil and water phases, different concentrations of PVA and PSAEG250, and varying centrifugation speeds. To investigate their influence on preventing agglomeration, lactose, a cryoprotectant, was added at different concentrations before freezing, while poly(ethylene glycol) (PEG) with varying molecular weights was introduced into the oil phase. The effects of freezing the particles using liquid nitrogen versus overnight freezing at -80℃ were also compared. The geometric diameter of the dried particles was measured using a laser particle analyser (MasterSizer 3000E). The feed pressure was set to 4 bars, and the feed rate was adjusted to achieve optimal obscuration values. Due to the large sample quantity required for the measurement, each measurement was performed only once.

Results and Discussion

The most optimal conditions were achieved with a 30 ml PVA solution at an 8% concentration, and a PSAEG250 concentration of 20 mg/ml in DCM, with 2 ml added to the PVA phase. Under these conditions, particle formation was less prone to collisions that might be the cause of agglomeration. Regarding lactose, a concentration of 1% proved to be the most effective in reducing particle agglomeration, granting about a 12% increase in number of MPs in the range 1-5 μm. The addition of PEG at different molecular weights to the oil phase had no significant impact on agglomeration. However, the freezing of the particles using liquid nitrogen prior to lyophilization, significantly reduced agglomeration, resulting in about 30% more particles within the 1-5 μm size range. Furthermore, as observed previously [4], the inclusion of Az improved the aerodynamic properties of the delivery system in a powder form, evident in a 10% increase in microparticles falling within the optimal size range for inhalation.

Conclusions

The introduced modifications resulted in the successful development of MPs that have the potential to serve as an effective drug delivery system for the lungs. These MPs could enhance targeted drug deposition, improving therapeutic outcomes for respiratory conditions.

Acknowledgements

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DUAL-FUNCTION COATINGS FORMED ON METAL SURFACES FOR MEDICAL PURPOSES

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Introduction

The development of medical therapy and diagnostics methods is associated with the increasing use of synthetic materials for direct contact with the patient's tissues. Such materials require specific, sometimes multi-stage approaches, that ensure the desired effect of the material on the patient's tissues and cells. Currently, there is a growing demand for multifunctional biomedical materials, which, in addition to biocompatibility, bioactivity, or antithrombogenic properties, will also be characterized by antibacterial activity. The search for new solutions for obtaining the surfaces with antibacterial properties concerns almost all synthetic surfaces intended for permanent or temporary contact with tissues. Therefore, Medicine needs multifunctional materials, because only such materials can lead to fully positive effects in therapeutic or diagnostic activities. Many strategies are applied for modifying the surface of biomedical materials in order to give them antibacterial properties [1]. Not only surface chemistry or modifications related to the release of specific substances with antibacterial activity, but also the creation of systems characterized by a specific nano or microtopography, are ways to create materials with desired biological properties [2]. One of the extremely attractive solutions in this area is creation of surfaces with hydrophobic/superhydrophobic properties. It has been shown that in addition to resistance to degradation and biocompatibility, materials described especially by Casie-Baxter theories are systems with antibacterial and anticoagulant properties [3]. Biocompatible multifunctional materials with antibacterial properties are of interest in fields such as orthopedics, dentistry, and otolaryngology. Both head and neck surgery (sinus and nasal surgery) uses a number of biomaterials such as; cochlear implants, middle ear prostheses, voice prostheses, which, besides bioactivity, should be characterized by antibacterial activity. On the other hand, materials such as tracheostomy tubes or various types of catheters, intended for diagnostics and periodic therapy, are devices that should primarily be resistant to the formation of biofilm and have properties that limit cell adhesion to their surface. Our experience clearly indicates that surface systems obtained by using siloxane sol modified with other sols and carbon or ceramic nanoparticles are a way to obtain coatings that meet medical requirements, especially in the area of cell adhesion control, creation of antibacterial and bioactive systems. The aim of the work was to study three types of sols, i.e. siloxane (Si), zirconium (Zr) and siloxane/zirconium (Si/Zr), intended for coatings on metals and to analyze their parameters, important for medical applications.

Materials and Methods

As part of the study, a number of coatings were prepared on the surface of medical steel. The coatings were obtained using the electrophoretic deposition method and the dip-coating method. After the deposition process, the materials were subjected to thermal treatment at 70°C. Three types of coatings were obtained using for this purpose, respectively: siloxane sol (Si), zirconium (Zr) and siloxane/zirconium (Si/Zr). Siloxane sol was prepared on the basis of the following precursors; Methyltriethoxysilane - Acros Organics, Dimethyldiethoxysilane, - Sigma Aldrich. Zirconium sol was made from tetrapropyl zirconate, Sigma Aldrich. The materials obtained in this way were tested in terms of physical and chemical parameters of the surface (SEM, EDS, FTIR, contact angle, surface energy). The results were correlated with parameters such as bioactivity and antibacterial activity. Selected samples were subjected to SBF tests and antibacterial tests using the quantitative method according to the JIS L 1902:2002 standard. The degree of bacterial reduction was determined. Selected samples were contacted with bacteria - Escherichia coli) and (Staphylococcus aureus). Two commercial strains of reference bacteria were used: S. aureus ATCC 6538P and E. coli ATCC 8 739. Degradation tests of coatings in artificial saliva *in vitro* for a period of 4 weeks were also carried out.

Results and Discussion

The coatings obtained using the three types of sols differed significantly in chemical structure, wettability and surface topography. The highest wetting angle was observed for the coating obtained from the Si/Zr sol – hydrophobic coating (102°), while the lowest one for the Si coating – hydrophilic coating (80°). It was shown that coatings obtained from the zirconium sol exhibit very good antibacterial activity only against Gram-negative bacteria, while the opposite situation occurs in case of coatings obtained from the siloxane sol. These coatings exhibited antibacterial activity only against Gram-positive bacteria. This means that both coatings obtained from the Si sol and coatings from the Zr sol exhibit satisfactory antibacterial properties, but only against one type of bacteria. On the other hand, coatings obtained from the Si/Zr – based sol exhibit antibacterial activity against both types of bacteria. The reduction factor for the coating obtained from Si/Zr sol is over 80%, while microscopic analysis in the live-dead test indicates bacteriostatic and bactericidal properties. This type of coating also has bioactive properties, as confirmed by incubation in SBF. EDS data indicate that significant amounts of phosphorus and calcium were observed only on the surface of coatings obtained from the Si/Zr sol. Coating degradation studies have shown their durability in *in vitro* conditions.

Conclusions

The results of our study confirmed the high usefulness of siloxane sol as a precursor of coatings for medical purposes. Modification of the sol, both in terms of the amount of single precursors and the addition of other sols, allows the manufacture of materials with a diverse chemical composition, topography, surface energy value, and surface charge. These parameters determine the type of biological response to the material.

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A NEW PROTOCOL FOR ANALYZING AIR FLOW RESTRICTIONS THROUGH INDIVIDUAL PROTECTION MASKS FFP CLASS

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Introduction

The effectiveness of individual protection masks, especially those classified under the Filtering Facepiece Particles (FFP) standards, is vital for ensuring protection against bacteria and viruses [1]. Traditional evaluation methods mainly focus on filtration efficiency and breathability, often neglecting the detailed analysis of airflow restrictions which can significantly impact user comfort and mask compliance [2,3]. This study introduces a novel protocol for analyzing air flow restrictions through FFP class masks using a vacuum pump and a digital anemometer. By integrating these tools, we aim to precisely measure and characterize the air flow dynamics, providing a more comprehensive assessment of mask performance. This approach not only ensures that the masks meet safety standards but also enhances the overall wearer experience by addressing factors related to airflow resistance.

Materials and Methods

Betulin was extracted from the bark of Betula species (birch trees) using a method involving Soxhlet extraction, which ensures high purity and retention of the compound's bioactive properties.

The suspension of betulin in ethanol was applied to the surface of medical-grade polypropylene masks using an ultrasonic spray coating system. This system operates at a frequency of 60 kHz, which facilitates the uniform application of the coating while maintaining the structural integrity of the masks. The ultrasonic spray coating process was optimized by adjusting parameters such as spray time, flow rate, and ultrasonic power to achieve a consistent and effective coating layer.

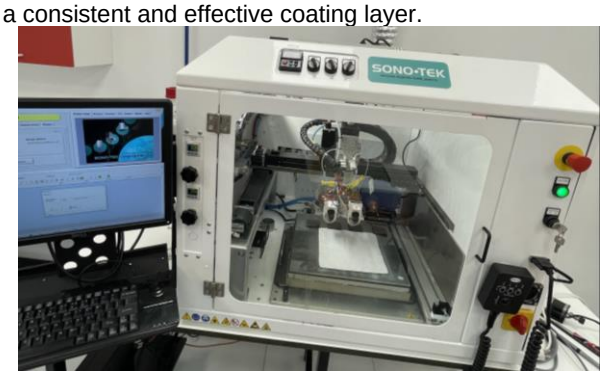

FIG. 1. Exactacoat Sono-Tek ultrasonic generator.

To evaluate the respiratory resistance and airflow restriction, an original testing apparatus was developed. This apparatus consists of a high-precision digital anemometer Benetech® GM8901) connected to a custom-designed airflow chamber that mimics human respiratory conditions. The device measures the pressure drop across the mask at various airflow rates, allowing for the precise determination of respiratory resistance.

The testing apparatus for measuring respiratory resistance and airflow restriction is a proprietary design that is pending patent protection. Therefore, detailed schematics and specific design parameters cannot be disclosed at this time.

Results and Discussion

According to our best knowledge, the application of the betulin coating significantly enhanced the antibacterial and antiviral properties of the masks, providing superior protection for the respiratory tract and preventing the formation of bacterial films. The proprietary device for measuring respiratory resistance demonstrated that the masks maintain optimal airflow while offering enhanced protection. Previous research confirms the antibacterial and antiviral effects of betulin. However, the use of sterilization using ethylene oxide increases the toxicity of the surface to microorganisms, including the tested strains of Staphylococcus aureus and Escherichia coli. This effect was also observed for other polymers, including PGA.

Conclusions

The innovative protective masks with ultrasound-applied betulin coating offer advanced respiratory protection by combining antibacterial and antiviral properties with low respiratory resistance. This development presents significant potential for both medical and personal protective applications. Additionally, after sterilization with ethylene oxide, it was observed by the provisions of the ISO 10993 standard: "A reduction in cell viability by more than 30% is considered a cytotoxic effect." Based on the cytotoxicity tests performed, cell survival is less than 50% for all tested samples.

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PREPARATION AND STABILITY TESTING OF MONOLAYER AND MULTILAYER SCAFFOLDS BASED ON METHACRYLATED HYDROGELS

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Introduction

Hydrogels are three-dimensional polymeric networks with the ability to retain and absorb enormous amounts of water. In recent years, they have emerged as a promising platform for tissue engineering in its native hydrogel form or in the processed form of dry porous scaffolds [1]. Modification of selected polymers with methacrylate groups enables fast hydrogel crosslinking initiated by irradiation caused by UV light in the presence of the photoinitiator [2]. Lithium phenyl-2,4,6-trimethylbenzoyl-phosphate (LAP) is an example of the photoinitiator used to initiate free radical chain polymerization upon light exposure [3]. Among other benefits, the primary advantage of this process is its high and precise controllability.

The goal of this study was to optimize the process for producing highly porous scaffolds from UV light crosslinked methacrylated hyaluronic acid, gelatine, and chitosan hydrogels and to determine their properties. Multilayered materials were made up of three layers with varying material compositions, while monolayered samples were made of methacrylated chitosan combined with either methacrylated hyaluronic acid or methacrylated gelatine.

Materials and Methods

All materials (Tintbionic®: CHIMA – methacrylated chitosan, HAMA – methacrylated hyaluronic acid and GELMA-80 – methacrylated gelatine, all from Polbionica, Poland) were prepared according to the specifications provided by the manufacturer. In brief, CHIMA was dissolved in 1% acetic acid, to yield 2% w/w solution, while HAMA and GELMA-80 were dissolved in phosphate buffered saline (PBS, Sigma-Aldrich, Germany) at concentrations of 2%, 5% and 10% w/w, respectively and placed in tubes wrapped in aluminium foil. Subsequently, the solutions were heated in a water bath to 50ºC for GELMA-80 and CHIMA or 35ºC for HAMA and vortexed every 2 min for 5 s. As 30 min passed, the photoinitiator LAP (Polbionica, Poland) was added, so its concentration in the solutions was 0.25% w/w and the solutions were heated in a water bath for another 15 min. Next, the pH values of the solutions were adjusted with 5M NaOH and 5M HCl to the values recommended by the manufacturer (pH=5.0-6.0 for CHIMA and HAMA and pH=7.0 for GELMA-80). For monolayered hydrogels, CHIMA solution was combined with HAMA and GELMA-80 in a 9:1 ratio. The pure CHIMA solution acted as a reference. The gelation process was then started by pouring solutions into Petri dishes and placing them under a UV lamp with the specified parameters (wavelength λ=405 nm, crosslinking time t=60 s). For multilayered hydrogels, only pure solutions of CHIMA, HAMA, and GELMA were used. Each layer was poured individually onto the Petri dishes and crosslinked as described above. Then another layer of different solutions of the methacrylated material was poured on top of the already crosslinked solution. As a result, the following multilayer samples were obtained: CHIMA_GELMA_HAMA, CHIMA_HAMA_GELMA, and HAMA_CHIMA_GELMA. As a next step, the samples were cut out using a circular puncher with a 12 mm diameter, put into a 24-well plate, and frozen at -80°C for 24 h, which was followed by the 48 h freeze-drying procedure (Alpha 1-2, Martin Christ, Osteroda am Harz, Germany). Microstructure, swelling properties, and the mass changes of the freeze-dried materials were tested as a function of incubation in PBS.

Results and Discussion

This study showed that it is possible to obtain multilayered hydrogels without using any adhesive materials in between the layers (FIG. 1). However, multilayered materials proved to have lower swelling properties, and they decreased the pH of PBS more drastically compared to monolayered materials. Such an observation can be explained by a higher dry mass of multilayered materials or a certain amount of uncrosslinked residues that could have been present in between layers. The mass of all the studied materials remained stable during the incubation of the samples in PBS. The addition of HAMA and GELMA-80 reduced swelling properties compared to the pure CHIMA scaffold, but on the other hand, it improved the stability of the monolayered hydrogels.

Conclusions

Multilayered materials proved to have lower swelling properties and they decreased the pH of PBS more drastically compared to monolayered materials. Such observation can be explained by a higher dry mass of multilayered materials or by the possibility that a certain amount of uncrosslinked residues could have been present in between layers. The mass of all the studied materials remained stable during the incubation of the samples in PBS. The addition of HAMA and GELMA-80 reduced the swelling properties of the scaffolds compared to reference monolayer CHIMA scaffolds, but on the other hand, improved their stability.

FIG. 1. Pictures of monolayered scaffolds (first row) and multilayered scaffolds (second row) before (left sample) and after freeze-drying (right sample).

Acknowledgements

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