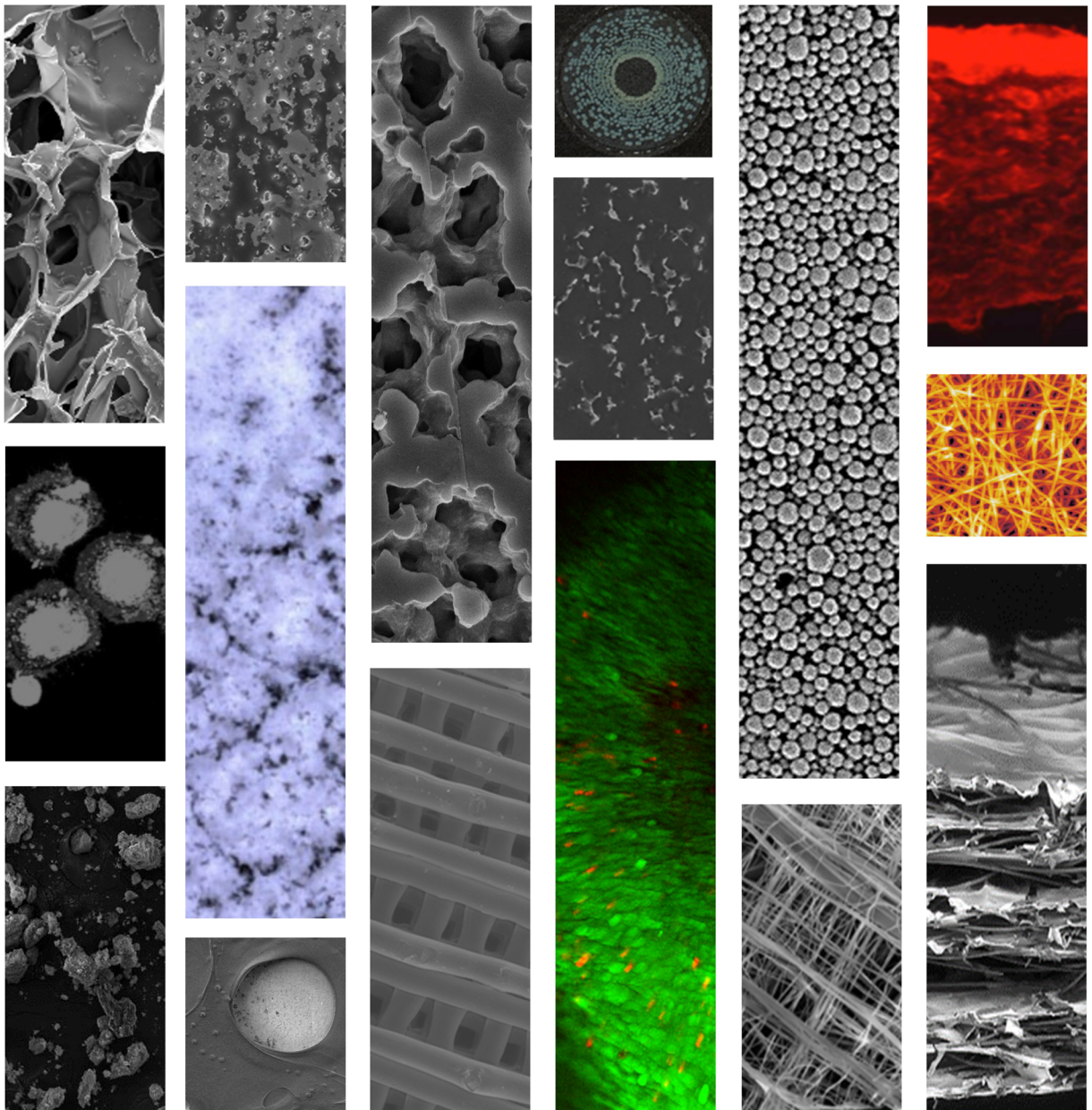
CONFERENCE PROCEEDINGS

32nd Biomaterials in Medicine and Veterinary Medicine

Annual Conference

12 – 15 October 2023 Rytro, Poland



Dear Colleagues and Friends,

This year's conference of the Polish Society for Biomaterials is dedicated to the memory of Doctor Emil Staszków – Founder of the Polish Society for Biomaterials, its first President and Honorary Member, the Initiator of the Engineering of Biomaterials Journal, a wonderful individual, our Friend.

Dr. Emil Staszków, MD, passed away on May 24, 2023. He was an outstanding surgeon and orthopaedist, a pioneer in the use of carbon biomaterials in reconstructive musculoskeletal surgery. He worked, among other places, at the Stefan Żeromski Hospital in Krakow, cared for generations of athletes in the Wisła Sports Society in Krakow, and was the founder and head of the Orthopedic and Trauma Department at the Gabriel Narutowicz Hospital in Krakow. Since the late 1980s, he was actively involved in research on the development of new carbon biomaterials, conducted at the AGH University of Krakow, and notably, he applied them with great success in clinical practice.

Dr. Emil Staszków was the driving force behind the establishment of the Polish Society for Biomaterials, an organization that brought together scientists, doctors, and entrepreneurs involved in the broad field of biomaterials. During the VI Conference on "Carbon and Ceramic Biomaterials" in Rytro in 1995, he organized the founding meeting and subsequently ensured the registration of the Polish Society for Biomaterials. During the first General Assembly of the PSB Members, held on May 18, 1996, he was unanimously elected as its President, a position he held continuously until 2013. The Society owes him also the tradition of the Krakow Christmas Eve Dinners, organized annually in December after the General Assembly of Members. For many years they have been, and still are, a wonderful way of integrating the PSB community and an excellent opportunity for closer, less formal interactions and the exchange of experiences and opinions.

The core, the scaffold, and the heart of the Polish Society for Biomaterials and all its activities are anchored to Doctor Emil Staszków. His ability to bring together representatives from various fields involved in biomaterials in Poland was unsurpassed.

Dr. Emil Staszków will be remembered for his optimism, dedication, visionary ideas and concepts, and his effective pursuit of set goals. A wonderful human being, always ready to help others. We are forever grateful for all of the lessons he taught us and will cherish his memory.

Scientific & Organizing Committee
32nd Annual Conference 'Biomaterials in Medicine and Veterinary Medicine'
12-15 October 2023, Rytro, Poland

Drogie Koleżanki i Koledzy,

Tegoroczna konferencja Polskiego Stowarzyszenia Biomateriałów jest poświęcona pamięci Dr. Emila Staszkowa – Założyciela Polskiego Stowarzyszenia Biomateriałów, jego pierwszego Prezesa i Członka Honorowego, inicjatora czasopisma „Inżynieria Biomateriałów/Engineering of Biomaterials", wspaniałego Człowieka i Przyjaciela.

Dr Emil Staszków zmarł 24 maja 2023r. Był wybitnym chirurgiem i ortopedą, pionierem w zastosowaniu biomateriałów węglowych w chirurgii rekonstrukcyjnej narządu ruchu. Pracował m.in. w Szpitalu im. Stefana Żeromskiego w Krakowie, opiekował się wieloma pokoleniami sportowców w Towarzystwie Sportowym Wisła w Krakowie, był założycielem i ordynatorem Oddziału Ortopedyczno-Urazowego Szpitala im. Gabriela Narutowicza w Krakowie. Od końca lat 80-tych XX w. angażował się w badania nad opracowaniem nowych biomateriałów węglowych, prowadzone w Akademii Górniczo-Hutniczej i – co należy podkreślić – z wielkimi sukcesami stosował je w praktyce klinicznej.

Dr Emil Staszków był inicjatorem powołania Polskiego Stowarzyszenia Biomateriałów jako organizacji skupiającej zarówno naukowców, lekarzy, a także przedsiębiorców związanych z szeroko pojętymi biomateriałami. Podczas VI Konferencji "Biomateriały Węglowe i Ceramiczne" w Rytrze w 1995 r. zorganizował zebranie założycielskie, a następnie doprowadził do wpisania Polskiego Stowarzyszenia Biomateriałów do Rejestru Stowarzyszeń. Na I Walnym Zebraniu Członków Polskiego Stowarzyszenia Biomateriałów, które odbyło się 18 maja 1996 r., został jednogłośnie wybrany na jego Prezesa, którą to funkcję pełnił nieprzerwanie do 2013 roku. To dzięki Jego inicjatywie tradycją stały się również Krakowskie Kolacje Wigilijne organizowane corocznie w grudniu po Walnym Zebraniu Członków naszego Stowarzyszenia. Są one dodatkowym elementem integrującym środowisko naukowe, a także doskonałą okazją do nawiązywania bliższych, mniej formalnych kontaktów oraz wymiany doświadczeń i opinii, co przekłada się na efektywne działanie całej naszej społeczności.

Istota, fundament i serce Polskiego Stowarzyszenia Biomateriałów są zakotwiczone w osobie Dr. Emila Staszkowa. Unikatowe były jego zdolności konsolidowania przedstawicieli różnych środowisk zajmujących się biomateriałami w Polsce.

Będziemy pamiętać Jego optymizm, zaangażowanie, wizjonerskie pomysły i idee oraz skuteczne działanie w dążeniu do postawionych celów. Przede wszystkim jednak Dr Emil Staszków był bardzo dobrym Człowiekiem, na którego zawsze można było liczyć, który nigdy nie odmawiał pomocy.

Będziemy pielęgnować pamięć o Nim.

Komitet Naukowy i Organizacyjny
XXXII Konferencji 'Biomaterials in Medicine and Veterinary Medicine'
12-15 października 2023, Rytro

32nd Annual Conference
Biomaterials in Medicine and Veterinary Medicine

12 - 15 October 2023 Rytro, Poland

ORGANIZING COMMITTEE

Elżbieta Pamuła - Chair of the Organizing Committee, President of the Polish Society for Biomaterials
Barbara Szaraniec - Secretary
Katarzyna Trała - Secretary of the Engineering of Biomaterials Journal
Patrycja Domalik-Pyzik - YSF representative, Website administrator

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SUBSTANTIVE SUPPORT

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
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POLISH SOCIETY FOR BIOMATERIALS

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SPONSORS & EXHIBITORS

CeCert is a dynamically developing certification and notified body offering its services in certification: management systems, medical devices, training, supplier audits. In 2019, we were entered on the list of authorized ERCA partners in the field of audit training, and from 2020 we have been accredited by the Polish Center for Accreditation for the QMS PN-EN ISO 9001: 2015-10 and MDMS PN-EN ISO 13485: 2016-04 programs. In October 2021, the Minister of Health authorized CeCert in the field of in-vitro diagnostic medical devices and appointed it as a notified body. On January 3, 2022, we were assigned the number of a notified body - 2934. We are currently trying to obtain notification in the scope of Regulation 2017/745 regarding medical devices.

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.

Foundation of Cardiac Surgery Development in Zabrze (FRK) was set up in 1991 in order to introduce the latest methods and techniques of rescuing human life into clinical practice, to support cardiac surgery and related fields, to support health protection and promotion activities. The Foundation's activity is in conformity with the Quality Management System as per ISO 9001:2015 in terms of research and implementation works, and training activity. The Medical Device Manufacturing Plant is a certified manufacturer of elements of heart prostheses. acc. to ISO 13485. The Heart Prosthesis Institute, as a sub-cell of the Foundation of Cardiac Surgery Development, is responsible for R&D work, especially for mechanical heart support and biocompatibility tests. The research area also includes planning and performing in-vitro and in-vivo biological biocompatibility tests of raw materials, biomaterials, and medical devices in the scope of the PN EN ISO 10993 standard.

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

● MEiN: 40 ● ICV: 100 ● ORCID ● DOI: 10.34821/eng.biomat. ● ISSN: 1429-7248 ● CC BY 4.0 license ●

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)
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- Cell & Tissue Engineering
- Biotechnology
- Chemical Engineering
- Nanotechnology
- Surfaces, Coatings & Films
- Medicine
- Veterinary Science

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CONTACT

Journal „Engineering of Biomaterials”
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SUBMISSIONS

Detailed information concerning manuscript preparation, reviewing procedure and journal policy are available at the journal's website:
www.biomaterials.pl

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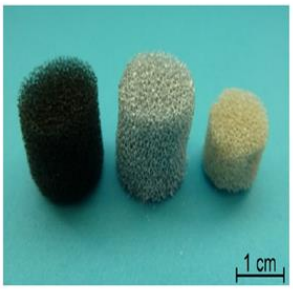
www.editorialsystem.com/biomaterials

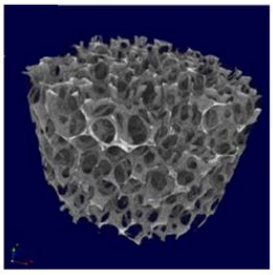
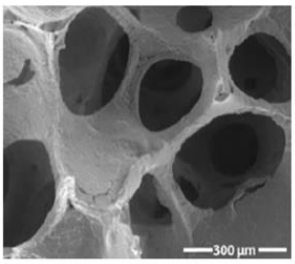
AKADEMIA GÓRNICZO-HUTNICZA
IM. STANISŁAWA STASZICA W KRAKOWIE
AGH UNIVERSITY OF SCIENCE AND TECHNOLOGY

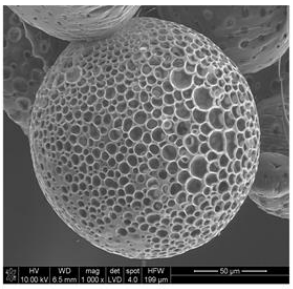
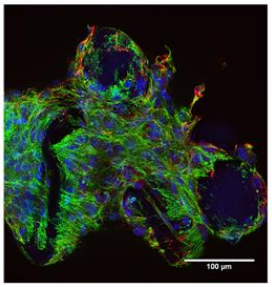
Patronat merytoryczny

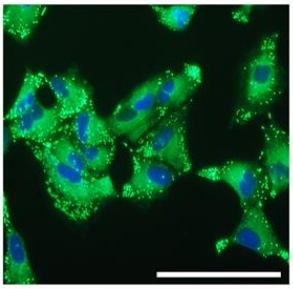
Studia Podyplomowe
„Biomateriały – Materiały dla Medycyny”

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.



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

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33rd Biomaterials in Medicine and Veterinary Medicine

Annual Conference

10 – 13 October 2024 Rytro, Poland

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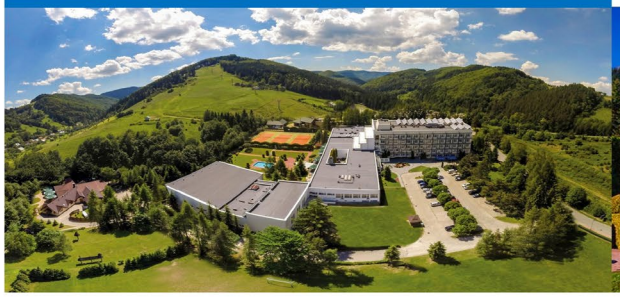
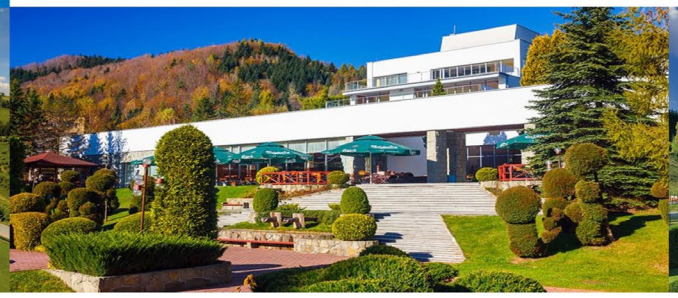
AN ABSTRACT





PROGRAM AT A GLANCE

Thursday, October 12

11:00	Departure to „Perła Południa” from Kraków
13:00 – 15:00	Lunch – Restaurant
15:15 – 15:30	**Welcome and opening remarks**
15:30 – 16:00	**In memory of PSB Founder Dr. Emil Staszków**
16:00 – 16:45	**Opening Lecture – Prof. Joachim Kohn**
16:45 – 17:15	Coffee break
	Session I
17:15 – 18:30	**Oral presentations**
19:00 – late	Regional Dinner – Cottage bar "Nad potokiem"

Friday, October 13

7:00 – 8:00	Swimming pool
7:00 – 8:30	Breakfast – restaurant
	Session II
8:30 – 9:15	**Plenary lecture – Prof. Aldo Boccaccini**
9:15 – 10:30	**Oral presentations**
10:30 – 11:00	Coffee break
	Session III
11:00 – 11:45	**Plenary lecture – Prof. Joachim Kohn**
11:45 – 12:45	**Oral presentations**
13:00 – 14:00	Lunch
	Session IV
14:15 – 15:00	**Plenary lecture – Prof. Werner E. G. Müller**
15:00 – 16:00	**Oral presentations**
16:00 – 16:15	Break
	Session V
16:15 – 16:45	**Keynote Lecture – Prof. Gary Bowlin**
16:45 – 17:30	**Oral presentations**
17:30 – 18:30	**Poster session and drinks**
19:00 – late	Dinner

Saturday, October 14

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

Sunday, October 15

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

DETAILED PROGRAM

Thursday, October 12

11:00	Departure to „Perła Południa” from Kraków
13:00 – 15:00	Lunch
15:15 – 15:30	**Welcome and opening remarks – Prof. Elżbieta Pamuła**
15:30 – 16:00	**In memory of PSB founder Dr. Emil Staszków** *(partially in Polish)*
16:00 – 16:45	**Opening Lecture – Prof. Joachim Kohn** *Biomaterials Research in Anticipation of Future Global Health Emergencies*
16:45 – 17:15	Coffee break

Session I chaired by Prof. Alina Sionkowska and Prof. Joachim Kohn

17:15 – 17:30	Martyna Sokołowska, Nina Kantor-Malujdy, Agnieszka Piegat, Judit E. Puskas, Marek Kowalczuk, Maria Letizia Focarete, **Mirosława El Fray** *Biomaterials and Sustainable Hospitals: Future Without a Way Back*
17:30 – 17:45	**Michał Wszoła** *3D-Bioprinted Tissue and Cancer Models – A Revolution in Preclinical Drug Research?*
17:45 – 18:00	Anna Sobczyk-Guzenda, Aleksandra Bednarek, Karolina Rosińska, Mateusz Bartniak, Adrianna Wierzbicka, Marta Kamińska, Marian Cłapa, Marta Kozakiewicz-Latała, Karol Nartowski, **Dorota Bociąga** *Biodegradable Blends of Polyesters for Filaments for 3D Printing of Stents*
18:00 – 18:15	Monika Mańkowska, Julia Semba, Filip Porzucek, Adam Mieloch, Anna Mleczko, Adam Augustyniak, Tomasz Szymański, **Jakub D. Rybka** *Advancing Meniscus Restoration: 3D Bioprinting with Novel Bioinks*
18:15 – 18:30	**Andrzej Swinarew,** Jadwiga Gabor, Paweł Raif, Grzegorz Brożek, Agnieszka Jarosińska, Jan E. Zejda, Szymon Skoczyński, Jarosław Paluch, Arkadiusz Stanula *Exhaled Air Metabolome Analysis for Childhood Asthma Fingerprints Identification*
19:00 – late	Regional Dinner – Cottage bar “Nad potokiem”

Friday, October 13

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

Session II chaired by Prof. Dorota Bociąga and Prof. Werner E. G. Müller

8:30 – 9:15	**Plenary lecture - Prof. Aldo Boccaccini** *Ion Releasing Bioactive Materials: Overview of Well-Known and Less Common Ions with Biological Activity for Tissue Regeneration*
9:15 – 9:30	**Timothy Douglas** *Whey Protein Isolate (WPI) as an Inexpensive Fibrillar Biomaterial Coating*
9:30 – 9:45	**Monika Śmigielska**, Piotr Jabłoński, Dominika Pawcenis, Roman Jędrzejczyk, Cristina Yus Argón, Manuel Arruebo, Agnieszka Kyzioł *Electrospun Silk Fibroin Composite Nanofibers with Metallic Nanoparticles and Bioactive Molecules*
9:45 – 10:00	**Júnio Augusto Rodrigues Pasqual**, Carla Cristina Schmitt Cavalheiro *Use Of Differents PVDF and Their Effects on The Properties of Composites Containing Photopolymerizable Resin and Hydroxyapatite*
10:00 – 10:15	**Julia Kulczyńska**, Roman Jędrzejczyk, Enrique Gamez Herrera, Manuel Arruebo, Agnieszka Kyzioł *Multicomponent Antibacterial Materials Containing Gold Nanopartciles, Natural Compounds and Antibiotics*
10:15 – 10:30	**Zhiyi Li**, Ihtesham ur Rehman, Timothy E.L. Douglas, Rebecca Shepherd *Fabrication and Evaluation of Pearl-Hydroxyapatite Composite Particles and Chitosan Based Scaffolds*
10:30 – 11:00	Coffee break – Poster viewing

Session III chaired by Prof. Mirosława El Fray and Prof. Aldo Boccaccini

11:00 – 11:45	**Plenary lecture - Prof. Joachim Kohn** *Novel Carriers Used in Topical Drug Delivery Systems*
11:45 – 12:00	Anna Karewicz, **Martyna Kasprzyk**, Daria Polakowska, Joanna Dulińska-Litewka, Czesław Kapusta *Spion Stabilized with Functionalized Polymeric Coatings for Anticancer Therapies*
12:00 – 12:15	**Adam Patalas**, Mariusz Sandomierski, Rafał Talar, Adam Voelkel *Mechanical Evaluation of Zeolite Coating on Titanium Alloy with Attached Drug*
12:15 – 12:30	**Ana Beatriz Sousa**, Cláudia Martins, Bruno Sarmento, Mário Adolfo Barbosa, Judite Novais Barbosa *Anti-Inflammatory Potential of Maresin-1 Loaded Zein Nanoparticles*
12:30 – 12:45	Jebarani J, **Ravichandran Kandaswamy**, Suvrochatterjee *Fabrication and Characterization of Functionalized PVA Buccal Film for Drug Delivery of Boswellia Seratta*
12:45 – 12:55	**Konrad Łuźniak** *A Medical Device on The Market - The Path From an Idea to Use in Patient*
13:00 – 14:00	Lunch

Session IV chaired by Prof. Aneta Zima and Prof. Gary Bowlin

14:15 – 15:00	**Plenary lecture - Prof. Werner E. G. Müller** *Physiological Inorganic Polymer Polyphosphate: The Key Driver of Bio-medical Regeneration Processes*
15:00 – 15:15	**Xiaohong Wang**, Werner E. G. Müller *Bio-silica and Bio-polyphosphate: Applications in Biomedicine (Bone Formation)*
15:15 – 15:30	**Susanne Staehlke**, Susanne Seemann, Manuela Dubs, Dirk Koczan, Hernando S. Salapare Iii, Arnaud Ponche, Annika Wartenberg, Thomas Seemann, Matthias Schnabelrauch, Karine Anselme, J. Barbara Nebe *Impact of Amino Group Density of Amine-Based Polymer Coatings on Cell Response*
15:30 – 15:45	Marlena Grodzicka, Marek Wiśniewski, **Aleksandra Radtke** *Fe/HA Biodegradable Scaffolds for The Needs of Modern Orthopaedics*
15:45 – 16:00	**Mikołaj Mielczarek**, Kamil Drożdż, Tomasz Gosiewski, Monika Brzychczy-Włoch, Tomasz Moskalewicz *Terpinen-4-Ol/Chitosan Coatings Developed by Electrophoretic Deposition: Microstructure and Surface Properties*
16:00 – 16:15	Break

Session V chaired by Prof. Xiaohong Wang and Prof. Piotr Niedzielski

16:15 – 16:45	**Keynote Lecture** Shannon E. Wallace, Alexandra E. Snyder, Adeline E. Nordmoe, **Gary L. Bowlin** *Mechanical Characterization of Near Field Electrospun Wind Angles for Bioresorbable Vascular Grafts*
16:45 – 17:00	**Lucie Svobodová**, Filip Koper, Agata Flis, Martina Trávníčková, Elżbieta Pamuła, Lucie Bačáková, Wiktor Kasprzyk *Novel Poly(Alkylene Citrate)-Based Materials for Vascular Tissue Engineering*
17:00 – 17:15	**Klaudia Cholewa**, Przemysław Kurtyka, Maciej Gawlikowski, Roman Major, Artur Kapis, Agnieszka Szuber-Dynia, Karolina Janiczak *Mechanical Circulatory Support for Children - Construction and Material Challenges*
17:15 – 17:30	**Sylwia Golba**, Sara Krawczyk, Kinga Góral, Izabela Matuła *Insight Into Influence of Sterylization Parameters on Polypyrrole Coating*
17:30 – 18:30	**Poster session and drinks**
19:00 – late	Dinner

Saturday, October 14

7:00 – 8:00	Breakfast – restaurant/Swimming pool
8:15 – 13:00	Excursion
13:00 – 14:00	Lunch
15:00 - 16:00	**YSF Rapid Fire Presentations chaired by Dr. Patrycja Domalik-Pyzik**
16:00 – 16:30	Coffee break

Session VI chaired by Prof. Agnieszka Sobczak-Kupiec and Prof. Krzysztof Pałka

16:30 – 16:45	Natalia Śmigiel-Gac, Anna Smola - Dmochowska, Katarzyna Jelonek, Monika Musiał - Kulik, Renata Barczyńska – Felusiak, Piotr Rychter, **Piotr Dobrzyński** *Antibacterial Activity and Cytotoxicity of Bioresorbable Polyesteramines Synthesised for Use in Biomedicine and Cosmetology*
16:45 – 17:00	**Piotr Piszczek**, Barbara Kubiak, Tadeusz Muzioł, Aleksandra Radtke, Patrycja Golińska, Tomasz Jędrzejewski, Sylwia Wrotek *Investigating the Bioactivity of Composite Films Incorporating Polymer Enriched with Titanium(IV)-Oxo Complexes.*
17:00 – 17:15	**Sylwester Domański**, Mateusz Urbańczyk, Agnieszka Zakrzewska, Marta Klak, Michał Wszoła *Non-Invasive Porosity Measurement of Commonly Used Biomaterials by Nuclear Magnetic Resonance*
17:15 – 17:30	**Ada Orłowska**, Joanna Jaworska, Wojciech Kajzer, Karolina Goldsztajn, Janusz Szewczenko *Development of a Method of Applying a Layer of Chitosan + Berberine on a PEO-Modified Spinal Implant Made of Ti6Al4V, Produced by SLM Method*
17:30 – 17:45	**Kamil Kleszcz**, Agnieszka Kyzioł, Roman Jędrzejczyk, Krzysztof Mars, Agnieszka Domka, Karol Kyzioł *Antibacterial Efficiency of Gold Nanoparticles/Gentamicin Hybrid System in Chitosan Layers*
17:45 – 18:00	**Marta Tuszyńska**, Łukasz Kaźmierski, Joanna Skopińska-Wiśniewska, Anna Bajek *Solvent Influence on Gelatin-Alginate Hydrogels Properties*
18:00 - 18:15	**Closing remarks and award session**
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BIOMATERIALS RESEARCH IN ANTICIPATION OF FUTURE GLOBAL HEALTH EMERGENCIES

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Introduction

Scientists have often warned about upcoming health emergencies. For example, back in the 1970's the rapid increase in the world's population resulted in warnings about wide-spread famine. In the 1980's the rapid increase of the number of diabetes patients triggered warnings of a global insulin shortage. The biotechnology revolution in agriculture mitigated the food shortage and the development of genetic engineering made it possible to obtain unlimited amounts of recombinant insulin. As in the famous story of "crying wolf", repeated false alarms dampen the response. When scientists repeatedly warned of the global threat of respiratory virus infections, the warnings were largely ignored. This contributed to the catastrophic death toll caused by COVID-19 world-wide.

Materials and Methods

This review is based on extensive literature searches and communications with scientists and leaders of all 10 major biomaterials societies. The opinions expressed in this lecture are fact-based and data-driven but are the subjective, personal opinions of the author.

Results and Discussion

Viral pandemics are an obvious threat. We are not only dealing with COVID, but a large number of viral diseases which are already major health threats or have the potential to become major health threats in the future. Biomaterials research (especially nanotechnologies) have greatly contributed to the development of mRNA vaccines and rapid detection kits. This line of research offers ample room for further innovation and improvement. The concept of intrinsically antiviral biomaterials was explored by G. Whitesides [1], but did not receive the attention this subject deserves now. This is a fascinating and most promising research area that was recently reviewed [2].

Antibiotic-resistant pathogens: The next pandemic could be caused by a virulent and transmissible bacterium. Scientists have warned repeatedly of this potential threat. There is a need to think beyond drug-based approaches. For example, silver and iodine are exceptionally powerful antimicrobial agents that are difficult to deliver and have therefore not reached their full clinical potential. Innovative material approaches have recently demonstrated significant promise and could open a new research direction for biomaterials science [3].

Anti-Fungal agents: Most people are unaware of the serious threat associated with fungal pathogens. Fungal infections are increasing at a rapid pace world-wide. Climate change has been identified as the probable cause for the increasing prevalence of invasive fungal infections. Fungi have evolved to grow at room temperature. The human body temperature is simply too hot for most fungal species to grow within our bodies. With increasing ambient temperatures, fungi have started to adapt to growing at higher temperatures, reducing our temperature safety margin and making the pre-emptive development of antifungal biomaterials even more important. The human mortality rate from invasive fungal infections is over 50%. Currently, there are only 4 classes of anti-fungal agents in clinical use. Creating a new anti-fungal agent is much more complicated and time consuming than developing an antibiotic. Fortunately, there are powerful biomaterials strategies to prevent fungal attachment and biofilm formation, but only a few laboratories focus on this important global threat. Two innovative approaches can be used: (a) the development of intrinsically anti-fungal polymers [4], and (b) the development of polymers that can prevent the adhesion of fungal cells to surfaces and their biofilm formation [5]. While 1 billion people currently experience food insecurity, fungi destroy 30% of the global food supply. Thus, intrinsically anti-fungal polymers will find wide applications not only in medicine but also in agriculture.

Biomaterials opportunities related to pollution and climate change: Global health threats are intertwined in many ways. For example, the massive use of personal protective equipment (PPE) has contributed to the formation of microparticles in the oceans. These microparticles are eaten by deep-sea amphipods which get poisoned, reducing global oceanic food chains and the amount of food available to humans world-wide. By creating degradable versions of PPE, innovative biomaterials can reduce the amount of medical plastic waste.

Drug delivery: New drugs are increasingly macromolecular biologics, requiring fundamentally new drug delivery systems. It seems that among the different branches of biomaterials research, nanotechnologies are most likely to become the dominant driver of future biomaterials research.

Conclusions

New global health threats are likely to emerge relating to diseases caused by antibiotic-resistant microbes, viral and fungal infections. Pollution and climate change threaten our food supply and health in new ways. In this time of rapid change, the research priorities of the biomaterial community will evolve. Some of the important future needs have been outlined here. This lecture is not meant to predict the future, but to open the discussion about how current research priorities may need to change to accommodate potential future health emergencies.

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ION RELEASING BIOACTIVE MATERIALS: OVERVIEW OF WELL-KNOWN AND LESS COMMON IONS WITH BIOLOGICAL ACTIVITY FOR TISSUE REGENERATION

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Bioactive glasses (BGs) are receiving increasing interest for tissue engineering (TE) applications, including both hard (bone) TE and soft tissue repair. Such applications are possible due to the biochemical reactivity of the BG surface which leads to time dependent interactions at the interface between BG surfaces and the biological environment. BGs are being designed with a wide variety of biologically active ions which are released to induce cellular responses of relevance for tissue regeneration. Such dissolution products of BGs are involved in several processes of new tissue growth, for example osteogenesis and mineralization in the case of bone tissue engineering [1]. Moreover numerous metal ions released from BGs have been shown to induce an angiogenic effect, e.g. in specific concentrations they enhance the secretion of vascular endothelial growth factor (VEGF) from stem cells. In addition, an emerging number of studies is considering the immunomodulatory effects of BGs in the framework of bone regeneration and wound healing [2]. In the first part of the lecture the general field of BGs in TE approaches will be discussed with focus on the interaction of biologically active ions (released from BGs) and stem cells leading to osteogenic and angiogenic effects. Due to its relevance, the effect of BGs on angiogenesis will be discussed in detail, showing results on different scaffold types and BG compositions.
The second part of the talk will focus on a series of less common ions (or even exotic ions) which are interesting to enhance the biological activity of BGs. Indeed, in addition to well-recognized biologically active ions that provide osteogenic, angiogenic, anti-inflammatory and antibacterial effects, such as zinc (Zn), magnesium (Mg), silver (Ag), strontium (Sr), gallium (Ga), fluorine (F), iron (Fe), cobalt (Co), boron (B), lithium (Li), titanium (Ti), and copper (Cu), a range of less common ions is being considered by researchers worldwide. For example, barium (Ba), bismuth (Bi), chromium (Cr), dysprosium (Dy), europium (Eu), gadolinium (Gd), ytterbium (Yb), thulium (Tm), germanium (Ge), holmium (Ho), iodine (I), lanthanum (La), manganese (Mn), molybdenum (Mo), nickel (Ni), niobium (Nb), nitrogen (N), palladium (Pd), rubidium (Rb), samarium (Sm), selenium (Se), tantalum (Ta), tellurium (Te), terbium (Tb), erbium (Er), tin (Sn), tungsten (W), vanadium (V), yttrium (Y) and zirconium (Zr) are being investigated [3]. The effect of incorporating such less-common elements in BGs will be covered with focus on tissue engineering applications.

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PHYSIOLOGICAL INORGANIC POLYMER POLYPHOSPHATE: THE KEY DRIVER OF BIO-MEDICAL REGENERATION PROCESSES

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Introduction

Inorganic polyphosphate (polyP) is one of the oldest bio-inorganic molecule, existing in biological systems. This physiological polymer is containing a much longer high-energy phosphate sequence than the universal energy donor adenosine triphosphate (ATP). PolyP has attracted increasing attention in the field of biomedicine since it promotes diverse metabolic and regulatory cell functions, especially in form of morphogenetically active nano/microparticles.

Materials and Methods

PolyP can be easily combined with other materials used in tissue engineering, e.g. for the production of bioprintable bio-inks or stable polymers such as polymethacrylate or polycaprolactone. Then, the material could be applied not only as a filler, but also for the fabrication of larger mechanically more stable implants [1-5].

Results and Discussion

The polyP nanoparticles transform physiologically into a coacervate phase, which is biologically active. In combination with other materials used in tissue engineering, for example, like with hydrogels, polyP is used as bioprintable bio-ink (even for cell printing) or as stable scaffolds (such as polymethacrylates of polycaprolactone). PolyP can be used not only as a filler (bone or teeth), but also as implants which are mechanically stable. Furthermore, together with negative polyanions, polyP self-organizes in the presence of divalent cations into stable polymer bundles, which are regeneratively active [6-9].

Together with suitable hydrogel-forming polymers and divalent cations, polyP is used for the fabrication of hybrid biomaterials with defined porosity and mechanical properties; they display *in vitro* and *in vivo* morphogenic activity (promoting cell growth, differentiation and migration).

A distinct property of polyP is to generate metabolic energy, which is utilized by the extracellular macromolecules to organize the complex extracellular matrix.

Recently, the physiological polymer polyP was disclosed as a member in the innate immune defense system, which prevents binding of SARS-CoV-2 to their target cells. In parallel, polyP reinforces the metabolism of the epithelial cells in the respiratory system. Additionally, polyP was shown to be a strong promoter of chronic wound healing [7,8,10].

Conclusions

With the discovery of polyP and the characterization of the multiple functions of this energy-rich biopolymer, a new physiological molecule has been introduced into the growing group of biomaterials of biomedical interest, which adds a novel principle: metabolic energy-delivery in addition to morphogenetic/regenerative activity. There is no other biomaterial that is provided with this property combination.

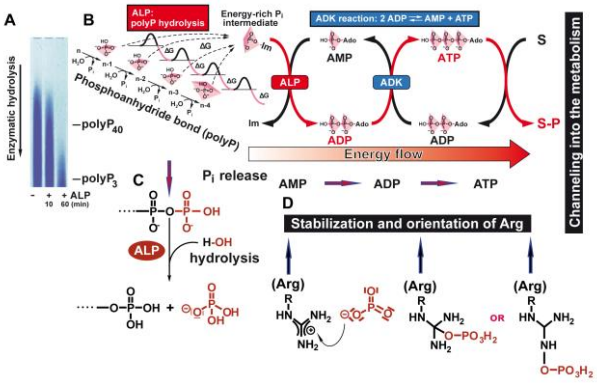

FIG. 1. PolyP as ATP generator and phosphate donor in the extracellular space. (A) SDS gel of polyP, hydrolysed by the alkaline phosphatase (ALP). (B) Storage of liberated Gibbs free energy (ΔG). The polymer is hydrolysed sequentially by the ALP und formation of ADP. Then the enzyme ADK catalyses the synthesis of ATP from ADP under liberation of AMP. Finally, ATP is channeled into the organismic metabolism. The activated P_i intermediate (metaphosphate) formed during cleavage of the phosphoanhydride bond is transferred (D) to the guanidinium side chain (Arg-P_i interaction). In this way, polyP binds to the surface of the SARS-CoV-2 spike protein.

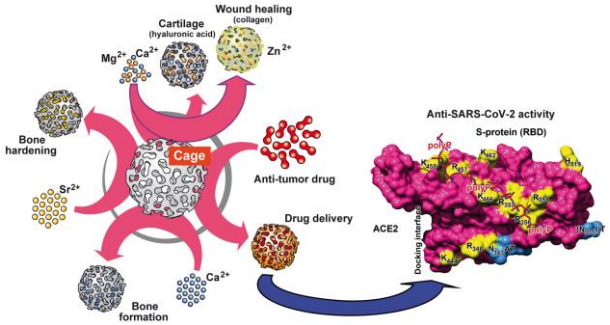

FIG. 2. PolyP as a genuine smart nano/micro biomaterial.

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NOVEL CARRIERS USED IN TOPICAL DRUG DELIVERY SYSTEMS

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Introduction

This plenary lecture will highlight the use of nanoparticles for the delivery of drugs into the skin or through the skin of a patient. Many scientists use the terms nanoparticle, nanocarrier or nanosphere interchangeably as long as the objects are between 1 and 1000 nm in size.

It is well known that nanoparticles are readily taken up by cells, using a wide range of different cell-uptake pathways. This makes nanoparticles useful drug delivery agents, but can also contribute to significant toxicity.

A highly undesirable group of micro- and nanoparticles are created by the degradation of plastic waste in water. These so-called microplastics are now distributed all over the globe and are probably present in every living organism, including humans.

In a perfect world, all nanoparticles would be biodegradable and designed in such a way that their only degradation products are naturally occurring nutrients, metabolites, or components known to be non-toxic. This requirement is satisfied by “TyroSpheres”, nanoparticles derived from the amino acid L-tyrosine, medium-chain fatty acids, and poly(ethylene glycol) (PEG).

TyroSpheres are “triblock copolymers”, consisting of a hydrophobic central core and two hydrophilic arms. TyroSpheres self-assemble when exposed to water and can form particles with a diameter that can be controlled within a range of 30 to 120 nm. Detailed studies have elucidated the structure of these nanoparticles, consisting of a hydrophobic core and a surrounding hydration shell (FIG. 1).

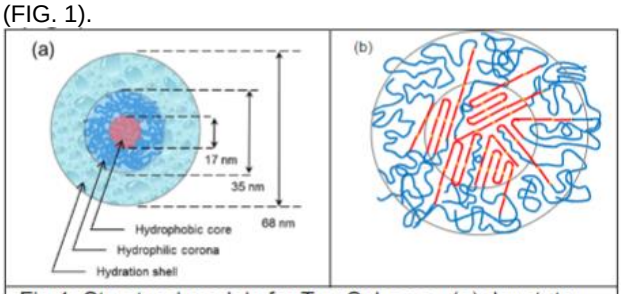

Fig 1: Structural models for TyroSpheres. (a) dry state; (b) wet, hydrated state.

TyroSpheres can readily deliver hydrophobic drugs through the stratum corneum (the uppermost layer of human skin) into skin, but not necessarily through the skin into systemic circulation. This is a significant advantage when targeting skin diseases that can be treated by topical drug delivery without requiring the systemic presence of drug. Acne is a well-known skin disorder. Adapalene is commonly used in the treatment of acne. In a collaboration with the laboratory of Prof Michniak-Kohn, we created adapalene-loaded TyroSpheres and conducted a number of preclinical test studies.

FIG. 2 illustrates the ability of TyroSpheres to deliver drugs into the deeper layers of human skin. Based these results, a series of studies was undertaken. Using pig skin samples in Franz diffusion cells, cryo-sectioning of skin followed by fluorescence microscopy showed that TyroSpheres are able to deliver adapalene within the hair follicles and epidermis.

To ensure clinical relevance of our studies, we compared the delivery of adapalene from a clinically used conventional crème formulation (Differin®.) into human cadaver skin and compared the permeation of adapalene against an experimental TyroSphere formulation, making sure that the applied dose of adapalene was identical in both treatment groups. The TyroSphere formulation resulted in an approximately 3-fold higher permeation of adapalene than the clinically used Differin®.

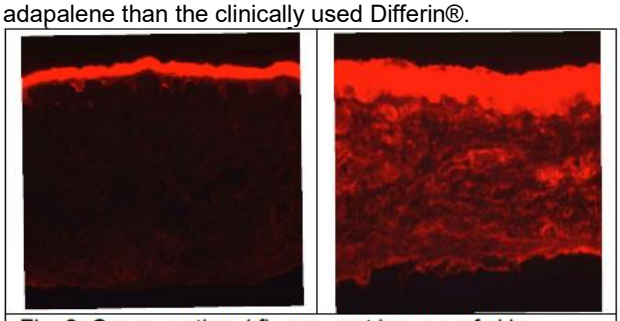

Fig. 2: Cross-sectional fluorescent images of skin samples after 6 hours. Left: Passive permeation of Nile Red in propylene glycol after 6 hours. All the drug remains on the surface of the skin. Right: TyroSphere delivery of Nile Red. The drug is visible throughout the dermis.

The endpoint of our study was the evaluation of the efficacy of adapalene-loaded TyroSpheres in a preclinical acne model. Adapalene TyroSpheres were formulated in a gel suitable for skin application. In vivo application to Rhino mouse skin (FIG. 3) allowed us to evaluate comedolytic and epidermal skin thickening effects of three formulations: (i) Differin® gel (delivering 35 µg/cm2), Adapalene TyroSpheres (ii) delivering either 20 µg/cm2, or (iii) 35 µg/cm2 of adapalene. We found that our oil- and alcohol-free aqueous gel of adapalene Tyrospheres delivering only 20 µg/cm2 was more effective than the clinically used Differin® gel in shrinking utricles in the rhino mouse acne model.

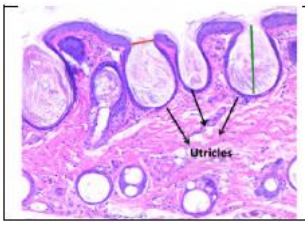

Fig 3: Histology showing utricles in rhino mouse skin. These utricles are a model for human comodones, the sites of acne pimples. Differin® is used clinically and delivers 35 µg/cm^2 of adapalene.

Conclusions

Nanotechnology is widely used in skin care products. In addition, some nanoparticles offer a new route of drug delivery into the skin of patients. TyroSpheres are nontoxic, biodegradable and environmentally safe. Here we showed that TyroSpheres can successfully encapsulate adapalene and release adapalene in a sustained manner. TyroSpheres were able to deliver their cargo to hair follicles and upper layers of skin. Small particle size in addition to good partitioning of adapalene in human sebum contributed to follicular delivery of the drug. Our work on the topical delivery of adapalene is a promising new approach for a wide range of topically active drugs.

Acknowledgments

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MECHANICAL CHARACTERIZATION OF NEAR FIELD ELECTROSPUN WIND ANGLES FOR BIORESORBABLE VASCULAR GRAFTS

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Introduction

Cardiovascular disease (CVD) is characterized by the thickening of blood vessels, atherosclerosis, and is an international health problem [1]. Treatments involve surgical procedures that implement either autologous or synthetic grafts to replace the diseased tissues. The goal of this experiment was to create a synthetic graft that could maintain its integrity long-term through tissue regeneration. Grafts were synthesized using a near-field electro spun (NFES) templates and printed at different angles (15°/75°, 20°/70°, 30°/60°, and 45°/45°). To test the mechanical properties of the synthetic bioresorbable biomaterial, the different angles were compared through mechanical tests such as ultimate tensile strength (UTS), percent elongation, Young's modulus for circumferential and longitudinal loads, suture retention, and burst pressure. These mechanical properties are crucial for testing whether the template can withstand body stresses. It was hypothesized that the larger templates angles would yield higher mechanical test values, therefore closer to the internal mammary artery (IMA) values.

Materials and Methods

A consumer 3D printer was modified as described in previous literature [2] to obtain degree angles of 15°/75°, 20°/70°, 30°/60°, and 45°/45° for vascular grafts, using a syringe pump with a 1 mL syringe and a blunt 23-gauge needle connected to the positive DC voltage source. Solutions were made of polydioxanone (PDO) dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) at concentrations of 100-112 mg/mL. 0.5 mL of the solution was loaded in the syringe and mounted vertically on the NFES print head, and a voltage of +1.6kV was applied to the needle with a polymer flow rate of 25 µL/h, an air gap of 2.2 mm, and an 80 mm/s translational velocity controlled by a customized G-code. The needle moved across a grounded 4 mm stainless steel mandrel controlled by a customized Q-code.

The wind angle of the templates was verified using scanning electron microscopy (SEM) to obtain images and the Windows 11 snipping tool and protractor function to measure the angles between the fibers (FIG. 1). Mechanical testing of the templates—UTS (longitudinal and circumferential), percent elongation (longitudinal and circumferential), suture retention, and burst pressure—was then performed until failure using a uniaxial testing frame in tension with a 25 lb_F load cell. Burst pressure was also measured using a syringe pump and transducer to record pressure at failure in mmHg. The circumferential and longitudinal Young's modulus was calculated using the ratio of tensile stress and tensile strain observed from the mechanical testing. The obtained values were then compared to IMA target values. Statistical analysis was performed with a significance of $p < 0.05$, and wind angle differences were evaluated using a one-way ANOVA and post hoc Tukey-Kramer analysis.

Results and Discussion

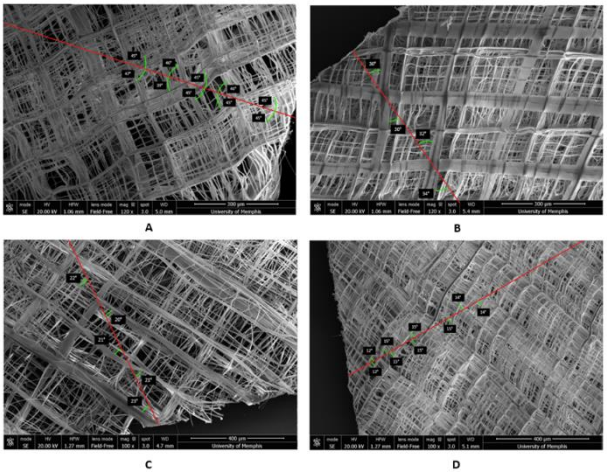

FIG. 1. Representative images from NFES for 45°/45° (A), 30°/60° (B), 20°/70° (C), and 15°/75° (D) wind angle representation (red indicates reference line, green indicates measured angle).

The results of the mechanical testing presented the 45°/45° angle grafts as the closest to the IMA requirements compared to the other wind angles; however, none of the templates met all the IMA target values. In ultimate tensile strength, all angles were short of the IMA values on the circumferential axis (4.1MPa) but were acceptable on the longitudinal axis (4.3 MPa). For percent elongation, the 20°/70° angle wind was significantly lower than the IMA value (134%) in the circumferential axis at 105 ± 25%, and all were significantly different for the longitudinal axis (59%). For the Young's Modulus, the 15°/75° angle wind was the only template significantly different (6.72±1.62 MPa) than the IMA circumferential axis value (8 MPa), while all the angle winds for the longitudinal axis were below the IMA target value (17 MPa). The suture retention, the 45°/45° was the only angle that was in the IMA target range (1.4-2.0 N) for a graft wall thickness of 0.15 mm and of 0.25 mm at 1.54±0.16 N. Finally, the burst pressure test showed that none of the angle vascular grafts were in range of the IMA (1600 mmHg) therefore not significantly different.

Conclusions

NFES bioresorbable vascular grafts show potential to promote artery function, making them a possible viable alternative to current grafts. Larger wind angles displayed mechanical properties comparable to IMA values indicated in literature. More testing is needed to investigate the effect wall thickness has on the mechanical properties of NFES grafts with different wind angles and if the mechanical properties of thicker grafts fluctuate at physiological states. The advancement of these NFES bioresorbable vascular grafts as an alternative to the current standard manufactured grafts could improve the success of small-diameter grafts.

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Dedicated to the memory of Professor Jan Chłopek.

BIOMATERIALS AND SUSTAINABLE HOSPITALS: FUTURE WITHOUT A WAY BACK

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Introduction

Biomaterials, such as medical silicone and poly(methyl methacrylate)(PMMA), have made significant contributions to the health and quality of life of modern generations. The search for biocompatible and reliable materials for use in medicine has most often turned to polymeric materials already existing on the market, such as the aforementioned silicone used for breast implants or PMMA, which has helped treat cataracts for decades.

The criterion of biocompatibility is unquestionably the most important in the development of materials for medicine, but the increasing focus on eco-friendly and sustainable technologies for the production of polymeric materials will undoubtedly revolutionize the market for medical devices, especially disposables or medical device packaging. The latest SARS-CoV-2 pandemic or the increase of laparoscopic procedures in developed countries are the best examples of enormous amount of medical waste production.

The WHO estimates that hospitals in high-income countries generate in excess of 3 kg of waste per bed per day [1]. A single hysterectomy procedure generates ~10 kg of waste for an abdominal procedure, with the vast majority of the waste being plastics [2]. Global demand for medical disposable products is forecast to grow 6.2% annually and reach a value of $273 billion in 2020, according to market report from the Freedonia Group [3]. With an aging society and rise in minimally invasive/robotic procedures, one can expect this issue of hospital waste to become more severe. Therefore, the solution to the problem is to develop a circular economy within the disposable medical device industry by developing new polymeric materials based on poly(butylene succinate) (PBS) copolymers that use monomers from renewable resources and are biodegradable or bioconvertable. They should also meet the demands of medical devices and packaging by facilitating—and maintaining—sterility, physical/chemical protection, ease of use (aseptic access).

Polyesters are currently one of the most important groups of materials as they exhibit several desirable features, including chemical groups susceptible to degradation which can be advantageous not only for biodegradable medical devices but also can be used for their packaging. Here, we will discuss the preparation and characterization of two series of segmented copolymers based on poly(butylene succinate)(PBS) and poly(butylene adipate)(PBA) as polymeric biomaterials for future sustainable hospitals, where biomaterial and its packaging is patient and environment friendly.

Materials and Methods

Poly(butylene succinate)-co-(dilinoleic succinate) (PBS-DLS), poly(butylene adipate)-co-(dilinoleic adipate) (PBA-DLA) and poly(butylene furanoate-co-dilinoleic furanoate) (PBF-DLF) which contain bio-based monomers were synthesized with variable segmental composition of hard to soft segments of 50:50, 70:30 and 90:10 wt%. Two series of block copolyesters with tunable properties was synthesized via solvent-free polycondensation method and were characterized using various analytical techniques, including nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), gel permeation chromatography (GPC), and dynamic mechanical analysis (DMTA). The *in vitro* cell compatibility of polymer extracts has been performed according ISO10993. Processing of polymeric materials has been performed with hot press to produce thin films and with injection moulding to demonstrate processability towards various shapes (plates, bars).

Results and Discussion

Expected chemical structure of synthesized copolymers was confirmed using ^{1}H NMR and FTIR analyses. DSC and DMTA measurements confirmed semicrystalline morphology of synthesized materials. The crystallization time (important for polymer processing) was dependent on the segmental composition for PBS series — the higher hard segments content, the better processability. The PBA series—due to low melting temperatures—was tested for coatings applications since all materials were soluble in benign solvents.

Conclusions

The obtained results confirmed the successful incorporation of different comonomers into the succinate- and adipate based copolymers. PBS and PBA-based copolymers had demonstrated their potential both for the medical device and packaging application thus providing a new material platform for both biomaterials and sustainable packaging.

Acknowledgments

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3D-BIOPRINTED TISSUE AND CANCER MODELS – A REVOLUTION IN PRECLINICAL DRUG RESEARCH?

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In recent years, intensive works have been carried out to develop alternative techniques that could replace the animals' usage in preclinical testing phase in the development of new drugs and therapies.
Changes in this topic are visible not only from the scientific point of view but, more importantly, also from the legal point of view. Until early 2023 (prior to the passing of the FDA Modernization Act 2.0 in December 2022), the US government required all investigational drugs to be tested on animals. This was a mandatory stage before starting clinical trials. Currently, US legislation allows for other nonclinical tests in preclinical drug testing that do not require the use of animals. Of course, the law does not completely ban animal testing. It only gives scientists a loophole and allows the use of scientifically proven testing methods without the use of animals. This is all the more important as studies show that up to 90% of generic drug candidates in clinical trials never reach the market.
So far, the most common animal models used in preclinical studies are mice and rats. Although it is often stated that they are a model similar in anatomical, genetic, and physiological terms to humans, their main advantage from the point of view of research is the ease of maintenance and size, as well as a short life cycle.
Although animal testing has helped people advance our scientific knowledge and develop new drug molecules, there is increasing talk that animal testing is not only costly but may also be ineffective. Especially when it comes to the transfer of results from animal models to the stage of clinical trials.
An example of this would be a medicine called fialuridine (for the treatment of hepatitis B). The results obtained in the animal model and in clinical trials are completely different. It turned out that this drug causes liver failure and is toxic to humans but not to mice.
The FDA's approval of the possibility of using alternative research methods, where justified and properly planned, should not have a negative impact on patients who will use them in the future (after the drug is launched on the market). In addition, the advantage of using validated models should be the shortening of the entire implementation process (the pre-clinical research phase) and the reduction of research costs.
From the point of view of pharmacology and medicine, the best alternative to the animal model seems to be miniature cell and tissue models, such as organs on a chip (OCC) and 3D bioprinting, which may additionally have a flow system. By using human cells for research in such models to imitate the functions and structures of organs, we get a simplified, faster-to-perform (compared to an animal model), and financially optimized model for drug screening and testing.

But what features should an ideal research model have? Currently, there are two most common models:
1. Organs on a chip
Microfluidic organs on a chip are small in size, which, thanks to the fact that they have human cells and microchannels imitating blood flow, allows for large-scale research at one time. The use of Organs on a chip is primarily a solution for cytotoxicity screening. According to the data, approximately 30% of drugs do not undergo toxicological tests at the stage of clinical trials. Although the results of preclinical studies using animal models were satisfactory. More and more experts recognize the potential of these systems, and thus they are becoming more popular at the stage of discovering and developing drugs.
2. Tissue bioprinting
Three-dimensional (3D) bioprinting of tissues is a milestone revolutionizing scientific research in the discovery and development of new active substances. An important issue that distinguishes 3D bioprinting is the possibility of recreating the microenvironment for a given type of cells or group of cells - the extracellular matrix. In addition, 3D bioprinting makes it possible for spatial cell growth. And it is known that cellular structures in 3D form provide environmental clues corresponding to those observed in physiological or pathological tissue. The third important point is the possibility of creating a vascular system, thanks to which it will be possible to remove metabolites and oxygen exchange from each part of the model to the same extent and to a degree similar to the processes occurring in the body.

To sum up, it is believed that both 3D bioprinting and OOC are already in use and will have an increasing impact on the development of the pharmaceutical market. Continuous development in the field of 3D bioprinting and chip systems provides new platforms for the development of a path that is supposed to reduce or even eliminate research on the animal model in the future. The 3D cell culture obtained by the bioprinting process mimics the spatial organisation of cells in a living organism. While OOC enables the monitoring of intercellular interactions. Therefore, testing the activity of drug candidates is more predictive and valuable. Therefore, in the long term, 3D bioprinting and OOC can be used as complementary tools, not competitive solutions.

Acknowledgments
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BIODEGRADABLE BLENDS OF POLYESTERS FOR FILAMENTS FOR 3D PRINTING OF STENTS

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Introduction

In tissue engineering, scaffolds play a key supporting role. Moreover, it allows to provide an appropriate environment for the functional reconstruction of the tissue. Increasingly, it is preferable that these systems degrade into harmless by-products within a given time frame. Biodegradable polyesters are widely used in this area [1,2]. Depending on their chemical structure and molecular weight, they have different physicomechanical and physicochemical properties. The period of possible use of these materials can be controlled by modifying the structure of the polymer chain and can range from several weeks to several years [3].
The aim of this work was to produce and characterize the physicochemical, mechanical and biological properties of a mixture of two thermoplastic, degradable biopolymers with a strictly defined chemical structure at various stages of their degradation. The described material is suitable for processing with additive technologies intended as a scaffold for the reconstruction of fragments of the urethra.

Materials and Methods

The tested material is a mixture of poly(lactide-co-glycolide) copolymer with polycaprolactone (PLGA/PCL). This material was mixed and extruded using a co-rotating twin-screw extruder Process 11. From the obtained filaments, stent printouts, with two different openwork geometries with dense and sparse arrangement of paths, were made. Degradation tests in saline solution at 37°C were carried out on all samples obtained. Changes in water absorption and mass loss of the obtained mixtures were analyzed. In addition, the chemical structure was tested on them by Fourier transform infrared spectroscopy (FTIR), changes in surface wettability, and deflection of the stents obtained using a tribotester. Cytotoxicity studies were performed using NIH/3T3 cells (ATCC CRL-1658). The exposure time was 24 hours, after which the MTT test was carried out in accordance with ISO 10993-5. For the material in the form of a filament, as well as after 3D printing, an assessment of platelet adhesion to the tested surfaces was also performed, along with an assessment of the degree of platelet activation and aggregation. Platelet adhesion was assessed by counting platelets visible on SEM images. The stages of platelet activation were analyzed according to Goodman's classification [4].

Results and Discussion

Two stent geometries were subjected to degradation tests in PBS solution at 37°C for 7 months. At 7 measurement points, tests were carried out to assess the chemical structure, changes in water absorption, weight loss and mechanical properties. Up to 1 month, no changes in the weight of all tested samples were observed. Only a weight loss of about 60% occurred after 2 months of keeping them in PBS solution. Then there was a decrease in the pH of the solution in which the samples were stored, and during the analysis of the FTIR spectra it was found a decrease in the intensity of the peaks from the ester bonds derived from PLGA and a significant increase in the intensity of the peaks belonging to the carboxyl groups. From the 2nd month to half a year, the mass loss was not as dynamic as in the first stage of the research, but in the macroscopic examination of the samples it was observed that after 7 months they lost their coherence after gentle shaking of the falcon with the PBS in which they were immersed. Within 6 months, there was also a very large increase in water absorption and it was most likely water entering between the polymer chains (or oligomeric forms) and permanently binding with them that caused such small mass losses of 35% to be recorded, and in fact the sample already lost the cohesion of the polymer matrix.
Due to the non-standard shape of the samples, in order to determine the stiffness of the material, it was necessary to independently develop a methodology for measuring this quantity. The stiffness of the printout structure was measured by compressing the stent of 1.5 mm length at a speed of 0.1 mm/s using a tribotester. The obtained data show that within a month of storage of 3D printed samples with two geometries in PBS, their mechanical parameters did not change. It was only after 2 months that an increase in deflection was observed, which is related to the penetration of water, cracking of polymer chains and the plasticization of the entire structure. At the same time, it should be emphasized that the increase in deflection in the case of stents with a denser structure is much less pronounced. The cytotoxicity study of the obtained printouts showed survival of NIH/3T3 cells >70% compared to the control what prove the biocompatibility of worked out blended biopolymer.

Conclusions

The obtained tests' results show that the addition of PCL to PLGA leads to get a stent that is more flexible. Printouts, regardless of the tested geometry, retain satisfactory mechanical properties for a period of one month. After this time, the mechanical properties deteriorate significantly and there is a rapid weight loss. This material is completely degraded after 7 months. This means that the degradation of PLGA significantly accelerates the degradation of PCL, which, due to its strong hydrophobicity, has much longer degradation times. In addition, the tested materials do not show signs of cytotoxicity and in extruded and printed forms are characterized by very low thrombogenicity – they do not cause platelet aggregation and activation in whole blood. It means that they can be considered as materials for biomedical applications.

Acknowledgments

The authors would like to thank the National Centre for Research and Development (Grant no: TECHMATSTRATEG/407770/2/NCBR/2020) for providing financial support to this project.

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ADVANCING MENISCUS RESTORATION: 3D BIOPRINTING WITH NOVEL BIOINKS

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Introduction

The meniscus has a limited capacity for self-healing and is vulnerable to injury as a result of the transmission of large loads and shear stresses within the knee [1]. According to clinical studies, meniscus injuries in the external zone have a higher chance of healing, whereas those in the internal zone frequently result in irreversible degeneration [2]. The goal of cutting-edge treatments should be to prevent surgery while preserving the mechanical properties and physiological functions of the meniscus. In order to greatly aid meniscus restoration, it is believed that therapeutic approaches like 3D bioprinting will be employed [3]. The scope of this study was preparation and evaluation of novel bioinks dedicated for meniscus implants 3D bioprinting.

Materials and Methods

Tested bioinks were composed of gelatin, alginate, carboxymethylated cellulose nanocrystals (CCNC), multiwalled carbon nanotubes (MWCNT) and/or decellularized extracellular matrix derived from porcine menisci (dECM). Constructs were bioprinted with extrusion-based approach. Rheological and mechanical properties of bioinks and bioprinted constructs were evaluated. For biocompatibility assessment XTT and LIVE/DEAD assays were employed. Finally gene expression of marker genes was evaluated with RT-PCR. For *in vitro* tests adipose-derived human mesenchymal stem cells (hMSC-AT) were used.

Results and Discussion

Our research proves that the extracted dECM provides good printability and printing accuracy when added to the bioink (FIG. 1).

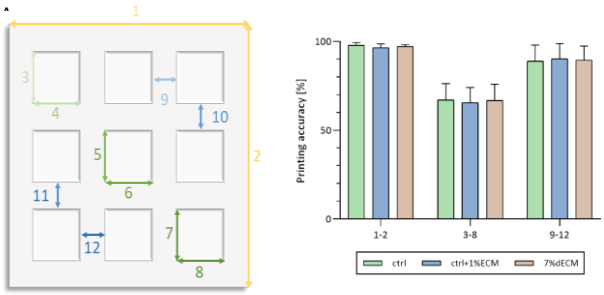

FIG. 1. Accuracy of 3D printing. Dimension measured for calculating the accuracy of 3D printing (on the left). Printing accuracy (on the right side).

Also has no adverse effect on cell survival in tested concentrations and suggest that hMSC-AT cultured in constructs supplemented with dECM are differentiating into fibrochondrocyte-like cells, typical for meniscus tissue (FIG. 2). What is more MWCN have reinforcing properties as proved with compression tests.

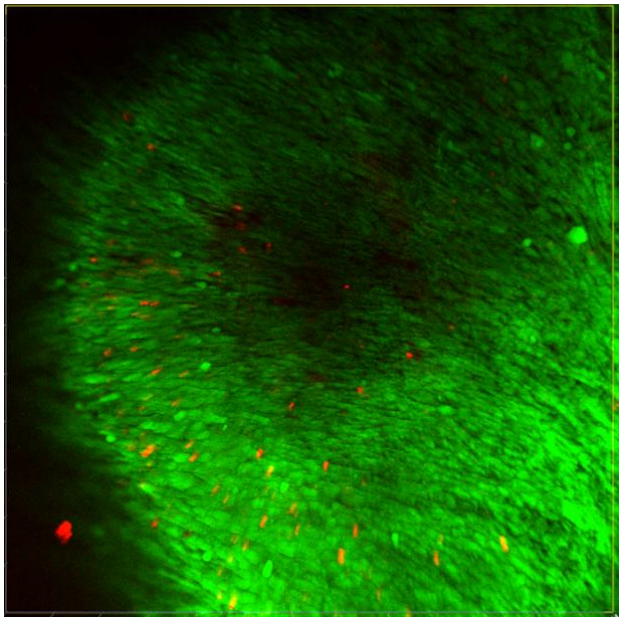

FIG. 2. hMSC-AT viability determined with the LIVE/DEAD assay. 3D projection of an exemplary stack of spheroids in the 7%dECM bioink at day 10 post-bioprinting.

Conclusions

A remarkable bioink component, decellularized extracellular matrix (dECM) closely resembles the environment of original tissue, including its structural, mechanical, and biomolecular characteristics. In this study, we propose usage of dECM derived from pig menisci as innovative bioink that is specifically designed for 3D bioprinting meniscal implants. We also evaluated bioink additive - MWCN which reinforces printed constructs.

Acknowledgments

This work was supported by the National Center for Research and Development TECHMATSTRATEGIII/ 0027/2019-00 grant.

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EXHALED AIR METABOLOME ANALYSIS FOR CHILDHOOD ASTHMA FINGERPRINTS IDENTIFICATION

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Introduction

The diagnostic accuracy of tests used in early diagnosis of childhood asthma is limited and could benefit from the application of noninvasive omics technologies, both in clinical and population-based setting.
This study is focused on highly porous organic materials to identify metabolic content of exhaled breath in asthmatic children and to develop an automatic classification method to measure metabolome changes including molecular mapping.

Materials and Methods

Group of 13 children (F/M: 6/7, mean age 8.8 ± 1.4 years) with diagnosed childhood asthma and 12 children (F/M: 6/6, mean age 9.5 ± 0.5 years) as control group were examined. The breath phase of all the subjects was collected using a highly porous aseptic material (patented device: holder PL230578, OHIM 002890789-0001). The specimens were analyzed using gas chromatography coupled with mass spectrometry (GC/MS). The algorithms of Spectral Clustering, KMeans, DBSCAN, and hierarchical clustering methods were applied in cluster analysis.

Results and Discussion

In asthmatic and not in control subjects the results of GC/MS showed the cluster of compounds including VOCS, SVOCS in the range of retention time from 12 to 30 min with the control peak of NOx, more apparent in asthmatic children (FIG. 1). The summary of the most characteristic signals for children with asthma is shown in (FIG. 2).

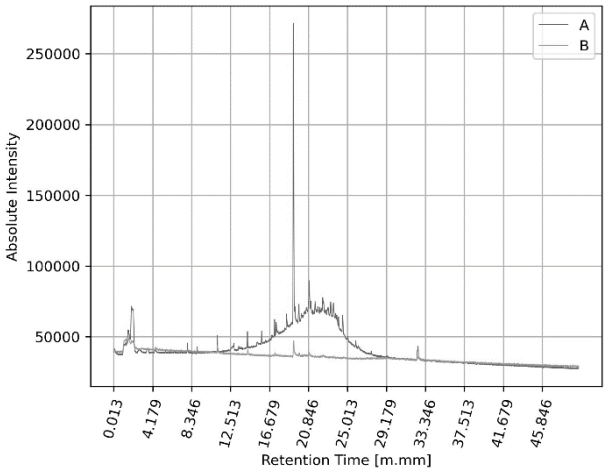

FIG. 1. Registered molecular fingerprint for healthy children B and for asthmatic children A.

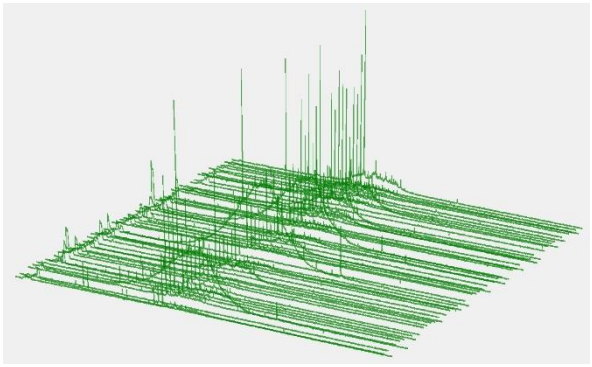

FIG. 2. Registered molecular fingerprint for asthmatic children A.

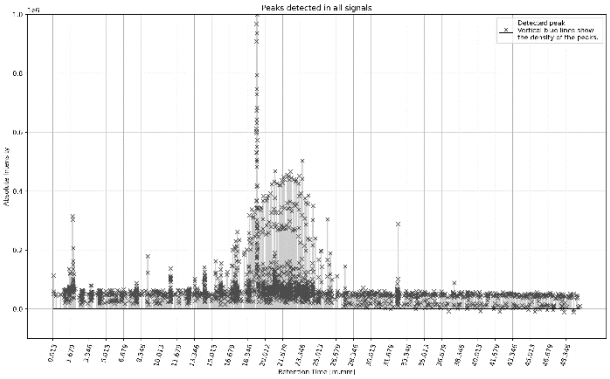

FIG. 3. The peaks indicated in the signal by the neural network recorded for asthma children

Conclusions

The use of GC/MS in analysis of metabolic content of exhaled air collected with novel porous polymeric material supported with neuron network peaks identification (FIG. 3) seems to offer a sensitive and differentiating method supporting screening for childhood asthma in clinical and population-based setting.

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WHEY PROTEIN ISOLATE (WPI) AS AN INEXPENSIVE FIBRILLAR BIOMATERIAL COATING

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Introduction

The interactions between a biomaterial and its surrounding biological environment are largely determined by the biomaterial's surface properties. Hence, there is a demand for surface modifications which promote the adhesion, proliferation and differentiation of cells. Additional important characteristics are cost, ease of adaptation to the topography and geometry of the substrate and sterilizability using commonly available and clinically acceptable sterilization methods, such as steam sterilization (autoclaving).

Whey Protein Isolate (WPI) is an inexpensive product of the dairy industry which consists of >95% protein, of which approximately three quarters is beta-lactoglobulin (β-LG) [1]. Previous work [2] showed that WPI in solution stimulates proliferation and differentiation of bone forming cells. WPI can form fibrillar suspensions upon stirring at high temperature [3] and have been found to remain stable in suspension for up to four months. Hence, it was hypothesized that coatings of fibrils formed from WPI would withstand autoclaving and would also promote the adhesion, proliferation and differentiation of bone forming cells. Furthermore, the formation of such coatings is very simple from a technical point of view and adaptable to any implant geometry; by bringing a biomaterial surface into contact with a WPI fibril suspension, fibrils can adsorb to the surface. Such fibril coatings can withstand autoclaving [4] and can serve as a matrix for the immobilization of other biologically active substances such as glycosaminoglycans (GAGs) such as heparin [5].

This presentation will review our previous work on WPI coatings [4,5] and describe plans for further research.

Materials and Methods

WPI fibrils were prepared according to the protocol described in [1]): WPI (BiPro, Davisco Foods International Inc., Eden Prairie, MN, USA) was dissolved in Milli-Q water to a final concentration of 2.5 wt% and the pH was set to 2.0 or 3.5 with 2M HCl. 40 mL of protein solution were heated at 90 °C for five hours under stirring at 350 rpm to allow the fibrillation reaction to take place. At the end of the specified time, the solution was immediately cooled on ice to stop the reaction.

The substrates, namely glass slides or Ti6Al4V discs (2 cm in diameter and 1 mm thick) additively manufactured in an A2 ARCAM EBM machine (ARCAM EBM, Mölnlycke, Sweden) were coated with fibrils by adsorption from the suspension. The substrates were left in contact with 1 mL of the fibril solution (2.5 wt%) for one hour, then rinsed three times with Milli-Q water to remove excess coating and left to air dry. For samples with heparin (MW: ~20000 Da) or tinzaparin (LMWH; MW: ~8000 Da), the protocol was repeated on the fibril-coated samples using a 10 wt% heparin (or tinzaparin) solution. Sterilization (121 °C, 15 min, 1 atm) was performed after the coating procedure. Addition of heparin or tinzaparin by adsorption from solutions was performed in sterile conditions.

In order to assess the biological performance of WPI fibril coatings, adhesion and proliferation of human bone marrow stem cells (hBMSC) were studied via confocal microscopy and metabolic activity by a metabolic assay, respectively. Osteogenic differentiation was assessed via the activity of the tissue non-specific alkaline phosphatase enzyme (TNAP) after 2 and 11 days in culture.

Results and Discussion

Importantly, WPI fibril coatings withstood sterilization by autoclaving. This was confirmed by Scanning Electron Microscopy (FIG. 1), contact angle measurements [4] and XPS measurements [5]. This is a significant advantage, as autoclaving is a widely available and clinically accepted sterilization method. The coating procedure is adaptable to different geometries and surface morphologies, including the rough surfaces of Ti6Al4V substrates. Cells adhered and proliferated on coated surfaces, both glass substrates and Ti6Al4V [4,5]. The presence of heparin and tinzaparin promoted the activity of TNAP after 11 days of culture [5]. This demonstrates that fibrillar coatings can serve as matrices for the immobilization of GAGs with biological activity.

Future work will focus on incorporation of other molecules with biological activity, such as polyphenols or phenolic molecules such as phloroglucinol, which has shown antibacterial activity as a component of hydrogels [6] and, as a component of collagen fibril coatings, enhanced expression of genes characteristic of osteogenic differentiation in SaOS-2 osteoblast-like cells while reducing inflammation markers in SaOS-2 cells and 3F3 fibroblast-like cells [7].

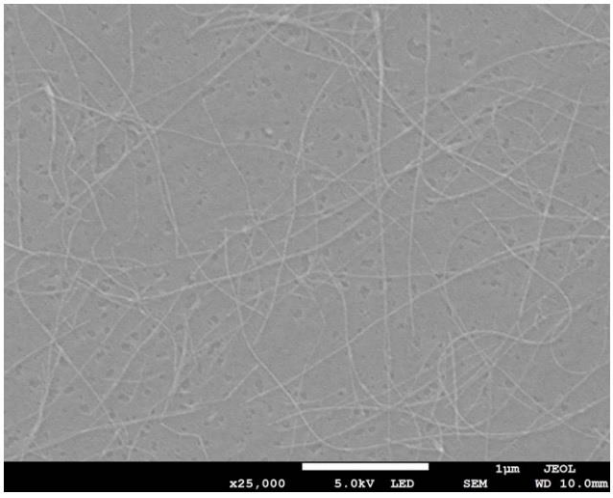

FIG. 1. SEM image of WPI fibrillar coating on glass slide after autoclave sterilization. Scale bar: 1 µm

Conclusions

WPI fibril coatings withstand autoclaving, allow adhesion and proliferation of hBMSC. The addition of heparin and tinzaparin to WPI coatings increases TNAP activity.

Acknowledgments

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ELECTROSPUN SILK FIBROIN COMPOSITE NANOFIBERS WITH METALLIC NANOPARTICLES AND BIOACTIVE MOLECULES

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Introduction

One of the leading causes of death worldwide is bacterial infections and growing antimicrobial resistance contributes to this serious problem. Chronic wounds or recurrent wounds can cause increased susceptibility to infection and even lead to sepsis and death. The use of nanotherapeutics can prove to be effective in hard-to-heal wounds [1]. Nanomedicine enables the fabrication of various types of materials for biomedical applications, among others, bandages or dressings for wound treatment and skin regeneration [2]. Nanofibers are characterized by a large active surface area and high porosity, which increases the permeability of the bandage to gases (oxygen) and the ability to absorb wound exudates. Moreover, the unique properties of this nanomaterial facilitate its loading with a large amount of drugs or wound healing accelerating agents [3]. Silk fibroin nanofibers can be obtained using the electrospinning technique and can serve as carrier matrices for biologically active compounds such as polyphenolic acids (*e.g.*, caffeic acid, CA) and metal nanoparticles (Ag NPs) for the management of infected topical wounds.

Materials and Methods

Electrospun fibroin nanofibers (SF_NFs) were decorated with metallic nanoparticles (SF_AgNPs) by inert gas condensation (IGC) and bioactive molecules *i.e.*, caffeic acid (SF_AgNPs_CA) were loaded by spin-coating deposition. The morphology, size, composition of the resulting materials and release of metal NPs/ions were precisely investigated by SEM, TEM, EDX, AFM, ICP-MS, ATR-IR. MIC (minimum inhibitory concentration) value for caffeic acid was determined for *Staphylococcus aureus* GFP. The antibacterial effect of SF_NFs, SF_AgNPs and SF_AgNPs_CA was tested *in vitro* on the following strains: *S. aureus*, *E. coli*, and *B. paramycoides* by serial dilutions in the culture medium and in direct contact.

Results and Discussion

Fabrication of composite SF_NFs involving Ag NPs and CA was optimized according to the rules of Sustainable Chemistry. The resulting SF_NFs were characterized by a smooth and homogeneous morphology with diameter around 470 nm (FIG. 1A). Ag NPs were sputtered on SF_NFs by the IGC technique with two different deposition times, resulting in fibroin nanofibers coated with Ag NPs (SF_AgNPs_1h, SF_AgNPs_2h). The size, morphology, and concentration of sputtered NPs were monitored by ICP-MS (*e.g.*, 1h: 20.5 ± 0.8 nm, 1.10 ± 0.03 μg/cm^2). Release of Ag NPs and Ag^+ ions was studied by ICP-MS after metal NPs and ions separation by dialysis (30 kDa cut-off centrifugal filtration tubes, 20 min, 4500 rpm, 4°C). Kinetic profiles of Ag NPs/ions release from the surface of the nanofibers (SF_AgNPs_1h) revealed a controlled release of the Ag(I) ions from the nanoparticles with almost no detection of released zerovalent Ag NPs (FIG. 1B). Whereas, in the case of SF_AgNPs_2h after 24h almost all Ag NPs were released from the mats (*ca.* 85%). This suggests that the sputtering time prolonged for 2 hours is too long for an efficient and homogenous covering of the SF_NFs (FIG. 1B). Uncontrolled and fast release of metal NPs may lead to toxicity and it is an undesired effect. Finally, the introduction of caffeic acid (MIC value for CA: 2.5 mg/mL, *S. aureus* GFP) resulted in the enhancement of the antibacterial effect towards both Gram-(+) and Gram-(-) bacteria in the direct contact approach. The resulting SF_AgNPs_1h_CA mats exhibited significantly higher antibacterial activity when compared with the corresponding SF_AgNPs_1h nanofibers (FIG. 1C-E).

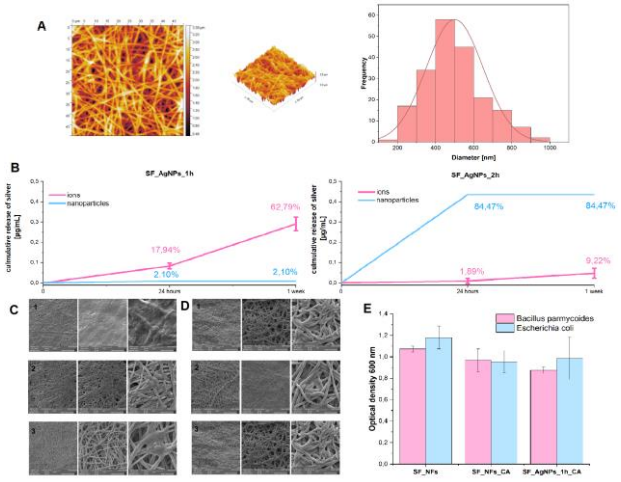

FIG. 1. A) AFM images of SF_NFs and histogram of fibers dimeter, B) the release of silver from SF_AgNPs_1h/2h, C) SEM images of *B. parmycoides* cultured and D) *E. coli* cultured on 1-SF_NFs_CA, 2-SF_AgNPs_1h, 3-SF_AgNPs_1h_CA, E) optical density analysis for SF_NFs, SF_NFs_CA, SF_AgNPs_1h_CA.

Conclusions

The resulting attractive electrospun materials which combine metallic nanoparticles and bioactive molecules are alternatives to currently used ineffective antibacterial wound dressings. Their multicomposite structure might overcome the problem of antimicrobial resistance by replacing the use of antibiotics for other antibacterial effective agents: Ag NPs altogether with natural compounds (caffeic acid). Noteworthy, the advantage of the proposed materials is the reduction of the used concentration of potentially cytotoxic Ag NPs by introducing the polyphenolic acid. Presumably, unknown additive/synergic effects contribute to the observed antibacterial effect *in vitro*.

Acknowledgments

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USE OF DIFFERENTS PVDF AND THEIR EFFECTS ON THE PROPERTIES OF COMPOSITES CONTAINING PHOTOPOLYMERIZABLE RESIN AND HYDROXYAPATITE

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Introduction

Advances in bone regeneration studies are stimulated by the increase in life expectancy of the population, which results in a greater occurrence of diseases related to bone tissue. The use of grafts, even though it is an approach with high employability when it comes to bone growth promotion methods, has some limitations such as the amount of material to be used and the acceptance by the organism.[1] Aiming to reduce problems in the bone regeneration process, the use of synthetic materials is an interesting and method due to the possibility of changing the properties of the desired product during the synthesis process. Some of the materials that we can mention that are applied as biomaterial are photopolymerizable resins[2], hydroxyapatite and polyvinylidene fluoride (PVDF).[1,2] These last two present interesting piezoelectric behavior for application as biomaterials due to the promotion of bone growth linked to this behavior.[2] In the work in question, effects of the presence of different types of PVDFs on the properties of the final resin/PVDF/HAp product were observed with a view to the use of this composite as a biomaterial in bone regeneration procedures.

Materials and Methods

The analyses for this study were performed for four types of PVDF, namely PVDF - PVDF1015/1001, PVDF6008/0001, PVDF11008/0003 and PVDF5140/0001. The TABLE 1 shows the properties of the four types of PVDF used in this work.

TABLE 1. General properties of PVDF types.

Sample	Monomer n°	ρ (g/cm³)	Tensile strength (Mpa)	T_g (°C)	Melting T (°C)
1015	Homopolymer	1,75 – 1,80	2100-2300	-40	171 - 175
5140		1,75 – 1,78	1000-1700		160 - 168
6008		1,75 – 1,80	1800-2500		170 - 175
11008	Copolymer	1,75 – 1,80	800-1200	-35	158 - 162

These materials were dissolved in dimethylacetamide (DMAc) to produce membranes. These materials were analysed with and without HAp obtained by wet synthesis in order to check the interaction of these two materials.

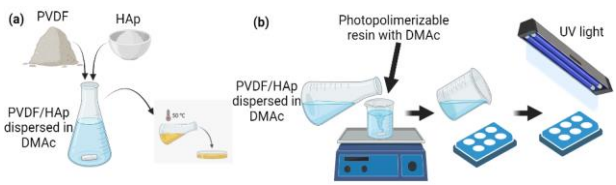

FIG. 1. Obtention of composite HAp/PVDF (a) and resin/PVDF/HAp (b).

In a second step, two types of PVDF (PVDF1015/1001 and PVDF6008/0001) and HAp were prepared to be added in commercial biocompatible photopolymerizable resin matrix to check the effect of this materials when the polymerization is the purpose. The composite ink was exposed to ultraviolet (UV) light for 90 seconds to ensure the cure of the material with any additive. The two steps can be seen in the FIG. 1.

The characteristics of the intermediate material and the final product were carried out using x-ray diffraction (DRX), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Shore-A hardness and viscosity.

Results and Discussion

The first characterization was made in PVDF dissolved in DMAc and commercial photopolymerized resin to analyse the viscosity in a rheometer. FIG. 2 shows the difference between the viscosity of the 4 types of PVDF. Because that PVDF1015 and PVDF6008 were chosen to do the preparation of the composite containing resin, PVDF and HAp due to the need not to have a very high viscosity considering the photo curing process and subsequent printing.

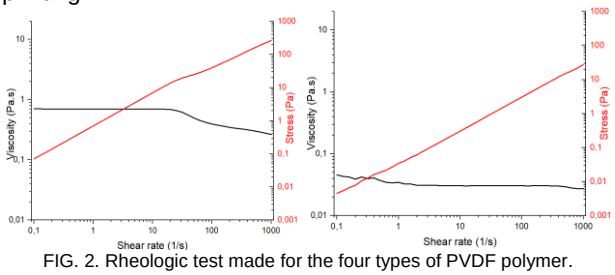

FIG. 2. Rheologic test made for the four types of PVDF polymer.

This difference of viscosity can also be observed when the three materials (resin, PVDF and HAp) are mixed in different temperature, fact influenced also by the initial viscosity of each type of PVDF and the temperature used during the photocure.

The presence of HAp can be observed in a dispersed mode in the PVDF/HAp composite. The FIG. 3 shows that condition to SEM images for PVDF1015.

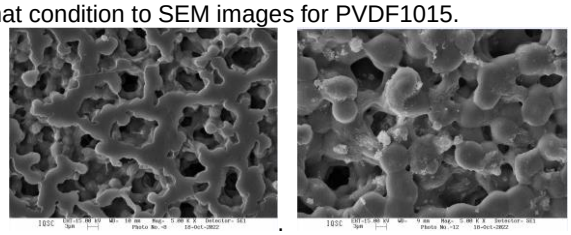

FIG. 3. SEM PVDF1015 with and without Hap.

Even in this conditions the increase of piezoelétric properties is expected because both this HAp and PVDF have this characteristic. The next step is to increase this behavior using functionalization.

In the final composite the observations were that this material have stability enough to be a composite and be used to 3D printing process. The Shore-A hardness showed was around 85,7 representening a good mechanical behavior and

Conclusions

The work shows the characterization of a promising biomaterial containing photopolimerizable resin, PVDF and HAp with good mechanical properties, good fillers distribution and with the possibility of increasing the piezoelectrical behaviour considering the presence of this properties in two of the three material in this composite,

Acknowledgments

Thank CAPES, CNPq, FAPESP and Rhodia.

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MULTICOMPONENT ANTIBACTERIAL MATERIALS CONTAINING GOLD NANOPARTCILES, NATURAL COMPOUNDS AND ANTIBIOTICS

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Introduction

The overuse and misuse of antibiotics has resulted in the emergence of antibiotic resistant pathogens in humans and animals, which is now recognized as a serious and global concern reaching alarming levels [1]. Infected postoperative or extensive burn wounds can be life-threatening and often lead to the development of sepsis because of the continued evolution of antimicrobial resistance (AMR). Even the new generation of antibiotics is virtually ineffective, and it is predicted that AMR will cause more deaths than cancer-associated diseases by the middle of the century [2]. Therefore the search for alternative antibacterial agents, having reduced chances to develop resistance, should be undertaken urgently. Sustainable chemistry, involving the synthesis of metal nanoparticles (NPs) by using natural compounds as reducing and stabilizing agents, is increasingly a popular eco-friendly synthesis method [3]. The antimicrobial action of natural components of plant extracts is well-documented, and moreover, there is demonstrated evidence corroborating the enhancement in the antimicrobial action of these components used in combination with other antimicrobial agents, both synthetic and natural (e.g., antibiotics) [4]. Thus, herein proposed, hybrid multicomponent materials involving Au NPs, *Rose* extracts, and antibiotics create the possibility of reaching unknown additive and/or synergy effects, which is a new approach in the fight against AMR.

Materials and Methods

The main components of *Rosa damascene* (RD) and *Rosa rugosa* (RR) extracts were defined by HPLC. Firstly, Au@RD NPs and Au@RR NPs were synthesized according to previously published protocols [3]. Then, procedures for the preparation of chitosan-based films containing: *i*) *Rose* extracts (RD, RR), *ii*) Au@RD NPs, *iii*) antibiotics (vancomycin (VAN) or gentamicin (GEN) against Gram(+) and Gram(-) strains, respectively), *iv*) mixtures of appropriate antibiotics and *Rose* extracts (RD_VAN, RD_GEN, RR_VAN, RR_GEN) were optimized. Films containing antibiotics alone served as controls for the tested multicomponent materials. In turn, films containing a mixture of the extract and the corresponding antibiotic were prepared to study the type of interaction between these components. Physicochemical characterization of the resulting materials was carried out by UV–vis, SEM, TEM, EDX, DLS, HPLC techniques. MIC (minimum inhibitory concentration) values for RD and RR extracts, Au@RD NPs, Au@RR NPs and antibiotics were determined for Methicillin-resistant *Staphylococcus aureus* (MRSA). Fractional Inhibitory Concentration Index (FICI) was determined for extracts, Au colloids, and antibiotic to assess possible interactions between these components. Finally, antibacterial effect *in vitro* for the resulting multicomponent films was examined on *E. coli* and *B. paramycoides* by the decimal dilutions method in the culture medium and by the disc-diffusion method.

Results and Discussion

Au@RD NPs and Au@RR NPs were synthesized according to synthetic routes complied with the rules of Sustainable Chemistry. The resulting NPs were characterized by having 17 nm (PDI = 0.1), 28 nm (PDI = 0.5) of diameter by TEM and by DLS (hydrodynamic diameter), respectively and -19 mV of Zeta potential. Polyphenols such as populin, quercitrin or catechin were identified by the HPLC analysis as the main components of RD and RR extracts. These compounds presumably are involved in the reduction of Au(III) ions, and in the stabilization of the forming nanostructures, and as well contribute to the observed biological effect *in vitro*. MIC values for RD, RR, Au@RD NPs, Au@RR NPs were determined as follows: 60% (v/v), 40% (v/v), 0.8 mg/mL and 1 mg/mL, respectively. An additive effect was observed for MRSA between RR and rifampicin by the FICI test. Antibacterial effects of chitosan films containing RD, RR, Au@RD NPs, Au@RR NPs, and/or selected antibiotic against *E. coli* and *B. paramycoides* was confirmed by reduction of both optical density and inhibition zones. Noteworthy, the most significant and prospective antibacterial effect was proved for the chitosan-based films containing RD extract and vancomycin (CSM_RD_VAN) when compared with the films containing only the antibiotic (41% reduction of optical density).

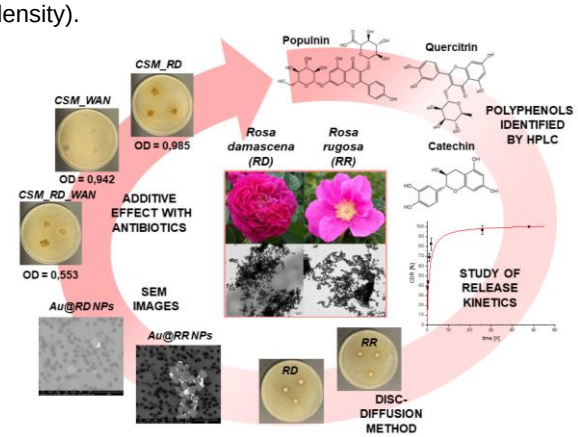

FIG. 1. Selected results from different experimental methods revealing the additive effect between *Rose* extracts and the antibiotic vancomycin.

Conclusions

In vitro tests proved the antibacterial action, exhibited by the reduced OD600 values and the increase in the zone of inhibition, of the studied hybrid nanosystems based on Au NPs and *Rose* extracts with antibiotics as a very promising antibacterial material to fight against AMR infections.

Acknowledgments

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FABRICATION AND EVALUATION OF PEARL-HYDROXYAPATITE COMPOSITE PARTICLES AND CHITOSAN BASED SCAFFOLDS

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Introduction

Chitosan and its derivatives have emerged as popular strategies in tissue engineering for bone repair, regeneration, drug delivery, and wound healing. Comprised of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues, chitosan possesses desirable properties including low toxicity, biocompatibility, biodegradability, and osteogenic potential [1]. However, the chemical imperfections of chitosan adversely affect its mechanical strength, posing challenges for designing bone regeneration scaffolds. In recent years, researchers have explored complex formulations of chitosan-based scaffolds incorporating specific particles to enhance osteogenesis and angiogenesis. Previous studies have shown that incorporating bioactive particles derived from stiff materials, such as ceramics, can improve the mechanical properties of the soft polymer [2-4].

In this study, the incorporation of pearl, hydroxyapatite (HA) and pearl/HA composites in chitosan scaffolds has been developed. Chemical, physical, mechanical, and biological properties of these bioactive composites were investigated by using Fourier transform infrared (FTIR), x-ray diffraction (XRD) scanning electron microscopy (SEM) and mechanical testing, and cytotoxicity study, respectively.

Materials and Methods

The acquired pearl/HA composite particles were synthesized via three different methods including precipitation method, hydrothermal technique, and sol-gel strategies. The methods used to prepare scaffolds were freeze-drying and freeze-gelation. Sole chitosan (CS), HA/CS, pearl/CS, and pearl/HA CS composite solutions were prepared and used to obtain the scaffolds.

Results and Discussion

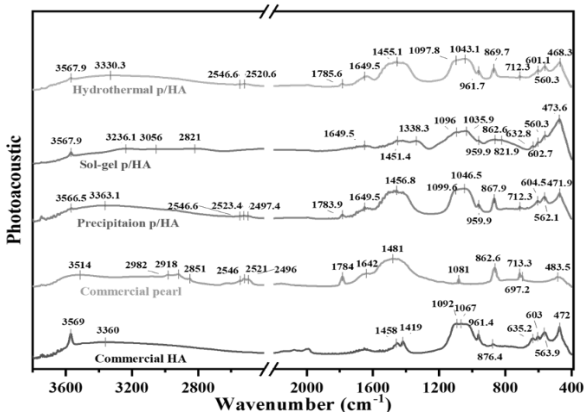

FIG. 1. Comparative FTIR-PAS results of synthesized HA/pearl particles.

XRD patterns and the infrared spectra confirmed the presence of HA and pearl powder in the resultant scaffolds, FTIR-PAS spectra provided more detailed chemical information of the composites, presence of PO_4 and CO_3 peaks, as well as amide peaks were clearly evident. Spectral peaks at 630 cm^{-1} and 873 cm^{-1} observed in precipitation and hydrothermal samples, indicated the potential chemical groups replacement between CO_3 and PO_4 in accordance with the previous study [5]. Further SEM studies in FIG. 2 provide evidence of attachment between HA and composite particles involved in chitosan scaffolds.

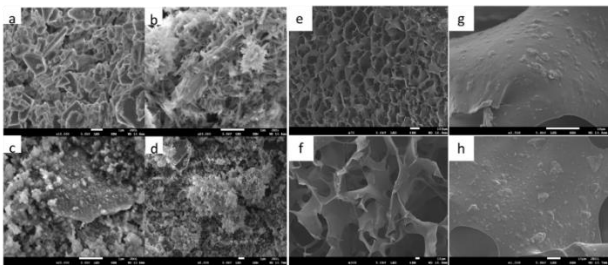

FIG. 2. SEM results of pearl, HA, and pearl/HA composite samples (a-d) and internal morphological studies of chitosan HA/p composite scaffolds (e-h). (a) the pearl particles at x10,000 magnification; (b) commercial non-sintered HA particles at x20,000 magnification; (c-d) pearl/HA composite particles at x20,000 and x5,000 magnifications, respectively; (e-h) cross-section of HA/p chitosan scaffold.

The results of Alamar blue (AB) assay confirmed the bioactivity of the composite scaffolds, and the higher growth of inflorescent reduction rate of the pearl/HA CS scaffold samples indirectly indicated an increased proliferation capacity of the pearl/HA composite particles compared to HA and pearl.

Conclusions

The synthesis of HA/pearl composite particles and their incorporation into chitosan scaffolds using freeze-drying and freeze-gelation methods has been carried out. SEM analysis confirms that the scaffolds possess suitable interconnectivity and porosity to support osteogenesis, as demonstrated by cell proliferation tests. Furthermore, the AB assay results demonstrate cell proliferation on the scaffolds, indirectly indicating the bioactivity of the pearl/HA composite particles.

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SPION STABILIZED WITH FUNCTIONALIZED POLYMERIC COATINGS FOR ANTICANCER THERAPIES

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Introduction

Superparamagnetic iron oxide nanoparticles (SPION) have recently gained a considerable interest in medicine due to their unique properties and biocompatibility [1]. They have found application in cancer therapies for diagnosis (as MRI contrasts), magnetic hyperthermia, and in the capture of circulating tumor cells (CTC). CTC are released from the primary tumor, typically as a result of the epithelial-to-mesenchymal (EMT) transition, upon which they gain the ability to migrate and invade other, even distant tissues. CTC are, thus, a cause of cancer metastasis, which is responsible for around 90 % of all cancer-related deaths. Our studies are focused on the preparation and optimization of the SPION-based systems for the effective magnetic capture and elimination of CTC. For this purpose, we have developed nanoparticles stabilized with cationic derivative of chitosan (CCh), providing a permanent positive charge, and with a pH-sensitive, bioactive conjugate of polyethyleneimine (PEI) and hyaluronic acid (HA). The nanoparticles were functionalized with specific antibodies and β-cyclodextrin-pioglitazone host-guest complex. The presence of HA allows the nanoparticles to interact with cancer cells via CD44 receptor on cells' surface while the specific antibody will bind to the target cell surface protein. Pioglitazone was proven to possess anticancer properties.

Materials and Methods

The CCh derivative of chitosan was obtained according to the procedure developed previously in our group [2] in reaction between chitosan dissolved in 1 % solution of acetic acid and glycidyltrimethylammonium chloride (GTMAC). A conjugate of PEI and HA was synthetized in aqueous conditions at pH=6 using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a carboxyl activating agent. The product was purified by dialysis and lyophilized. Both CCh and PEI-HA were analysed by ^{1}H NMR, ATR-FTIR and elemental analysis. SPION/CCh and SPION/PEI-HA systems were obtained by co-precipitation of iron (II) and (III) salts in the presence of ammonia and CCh or PEI-HA. The nanoparticles were purified by magnetic filtration and stored as buffered suspensions at 4°C. Nanoparticles formed by covalent binding of HA to the surface of the SPION/PEI system were also obtained, where HA was grafted using EDC. Iron content of the suspensions was determined based on the reduction of iron (III) to iron (II) and formation of the complex with 1,10- phenanthroline. Average size and zeta potential of the obtained particles were measured by DLS/ELS and morphology was evaluated by AFM. Anti-VCAM-1 antibody was attached either using previously introduced tosyl groups or by EDC/NHS chemistry. Tosylation was performed in pyridine using tosyl chloride. The successful attachment of the antibody was confirmed by immunostaining with a secondary antibody bearing Alexa Fluor 674 fluorophore. β-cyclodextrin (BCD) was modified with p-toluenesulfonyl chloride to introduce reactive tosyl groups. BCD-ToS was then characterized using ATR-FTIR and ^{1}H NMR. The inclusion complex of BCD-ToS with PIO was synthesized based on modification of the reported procedure [3]. BCD-ToS was further attached to SPION/CCh, and PIO was complexed under previously established conditions. The obtained system was characterized using DLS and ATR-FTIR. Magnetic properties of the nanoparticles were studied using Mössbauer spectroscopy and Vibrating Sample Magnetometry.

Results and Discussion

CCh and PEI-HA, and SPION systems based on these polymers were successfully synthetized. SPION/CCh nanoparticles had an average size of 120 nm and zeta potential of +47 mV. SPION/PEI-HA had an average hydrodynamic diameter of 230 nm and zeta potential of +56.5 mV. Both types of nanoparticles were therefore colloidally stable. Based on AFM studies nanoparticles were spherical in shape. SPION/PEI/HA system was also successfully obtained, with an average size of 253 nm and the zeta potential of -33.7 mV. They were, therefore, also colloidally stable, but showed a slightly higher tendency to aggregation. Anti-VCAM-1 antibody was successfully attached to the obtained nanoparticulate systems. β-cyclodextrin was successfully tosylated, and the degree of tosylation was estimated as 97%. Stoichiometry of the formed β-cyclodextrin-pioglitazone complex was also determined as 1:1. The complex was successfully attached to the SPION/CCh surface.

Conclusions

We have obtained and optimized the synthesis of three SPION systems stabilized with cationic derivative of chitosan, PEI-HA conjugate and the bilayer of PEI and HA. The obtained nanoparticles were colloidally stable, had low dispersity (low polydispersity index) and size in the range of 120-250 nm. We have also successfully attached anti-VCAM-1 antibody allowing for the specific interactions with cancer cells. β-cyclodextrin-pioglitazone complex was obtained, studied, and attached to the surface of SPION/CCh. Superparamagnetic properties of all obtained systems were also confirmed, allowing for magnetic targeting and capture.

Acknowledgments

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MECHANICAL EVALUATION OF ZEOLITE COATING ON TITANIUM ALLOY WITH ATTACHED DRUG

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Introduction

Due to injuries and bone diseases, numerous implant surgeries are performed annually worldwide. Zeolites, versatile materials utilized in various biomedical applications, including drug delivery, have shown promise in modifying titanium alloys. Zeolite coatings have improved oseointegration, osteogenesis, and bone regeneration. Furthermore, they eliminated cytotoxic ion release, exhibited good biocompatibility, and possessed antibacterial properties. The potential of zeolite layers in implantology is evident, but efforts should continue to enhance their properties. Incorporating magnesium, calcium or strontium ions via ion exchange can improve osseointegration, bone resorption inhibition, and bone formation promotion. Hydroxyapatite layers are commonly used for releasing strontium ions, but other modifications with bioactive glass, TiO_2 nanotube, and strontium titanate have been explored. Bisphosphonates, ideal for supporting osseointegration, can be delivered from zeolite layers due to their strong interactions with divalent ions. Controlled release of bisphosphonates is crucial to avoid inflammation and promote wound healing. Additionally, the tribological evaluation focuses on the biotribological behaviour of zeolite coatings on Ti-6Al-4V alloy implants in contact with cortical bone tissues. The mechanical properties of the modified surfaces are assessed to optimize coating performance in prostheses. The study presented here developed zeolite layers containing magnesium, calcium or strontium ions and investigated their potential as drug carriers for osteoporosis treatment, analysing drug sorption and release kinetics.

Materials and Methods

To assess drug sorption, each modified titanium sample was immersed in a pre-prepared drug solution (0.4 mg of risedronate dissolved in 2 mL of 0.1 M TRIS-HCl solution at pH 7.4) for a duration of 7 days. The concentrations of risedronate in the solutions were measured using UV-Vis spectroscopy. Following risedronate sorption, the modified samples were exposed to 1 mL of SBF and maintained at a controlled temperature of 36.6 °C. Optical surface profilometry was carried out using Bruker Alicona RL apparatus, which is an optical 3D measurement system containing a microscope and profiler.

Nanoindentation measurements were made with a Picodentor HM500 (Fisher, Sindelfingen, Germany) device according to the ISO 14577-1 standard. Ten nanoindentation tests were performed for each sample.

Results and Discussion

All materials underwent microscopic characterization. Upon analyzing the images, it became evident that a porous surface was achieved on all samples, affirming the successful modification with a zeolite coating. The absence of observable alterations after drug sorpsion suggests that the drug most likely adhered via ions rather than precipitating onto the modified titanium surface. The attainment of a surface with precisely pore sizes offers numerous advantages for regenerative processes. Excessively large pore sizes and inconsistent pore distribution may heighten the risk of damage and cracking in the resultant layer. From the nanoindentation tests carried out, it can be seen that SrZeo is characterized by better mechanical properties.

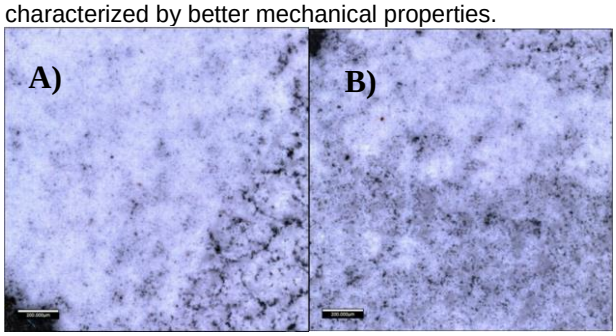

FIG. 1. The Mg (A) and Ca (B) zeolite layers.

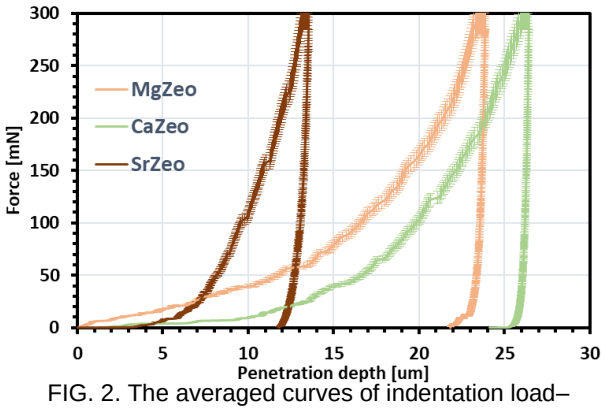

FIG. 2. The averaged curves of indentation load–indentation depth for each type of layer.

Conclusions

The Ti6Al4V was effectively modified with zeolite layers containing divalent ions. Analyses were conducted to characterize the resulting coatings and validate the attachment of the drug (risedronate) to the modified surfaces, confirming our assumptions. The formation of porous zeolite layers on the titanium surfaces was successfully confirmed. These appropriately sized pores hold the potential to enhance tissue regeneration around the implant when covered with the obtained material. By leveraging various divalent ions, we can influence the drug release profile and precisely control the time it takes for the drug, facilitated by ion exchange, to be released directly to the affected area of the body.

Furthermore, the higher estimated strength of the SrZeo coating suggests that it is unlikely to sustain damage during the mechanical fixation of the endoprosthesis within the bone. The gradual release of the drug over several months paves the way for the development of endoprostheses that not only replace weakened bone but also have the potential to inhibit the further progression of osteoporosis in their vicinity.

Acknowledgments

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ANTI-INFLAMMATORY POTENTIAL OF MARESIN-1 LOADED ZEIN NANOPARTICLES

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Introduction

The incidence of chronic wounds is a problem escalating worldwide. The associated healing process is especially problematic in population that are aging and show increased morbidity [1]. Therefore, the search for novel strategies that will improve tissue repair is at the cutting edge of regenerative engineering.
The main goal of this research work is to develop an innovative strategy consisting of zein monodisperse nanoparticles loaded with maresin-1 to induce a pro-regenerative microenvironment that will ultimately accelerate wound healing.
Zein is an insoluble prolamin protein that is extracted from corn [2]. Maresin-1 (Mar1) is a potent immunoresolvent, biosynthesized in inflammatory exudates to control inflammation via stimulating resolution programs through limiting polymorphonuclear leukocytes infiltration and enhancing macrophage uptake of these apoptotic cells [3].

Materials and Methods

The Dolomite Microfluidics® chip was used as platform to load maresin-1 into zein nanoparticles. The organic phase consisted of a mixture of zein and maresin-1 in 70% ethanol, whereas the aqueous phase consisted of Milli-Q water. The nanoparticles were produced by flow-focusing the organic central stream with the aqueous outer fluid. The final solution was magnetically stirred for 3h at RT (21ºC) and centrifuged for 40 min through a filter device with a molecular weight cutoff of 30kDa, at 2000g. Liquid suspensions of unloaded and maresin-1 loaded zein nanoparticles were stored at 4°C for 30 days after production. The particle size, size distribution, and zeta-potential were assessed. The ability of maresin-1 loaded zein nanoparticles to affect cell viability was assessed in primary human macrophages. The capacity of the nanoparticles to affect macrophage polarization was also evaluated.

Results and Discussion

Zein nanoparticles loaded with maresin-1 presented average diameter values in the range of 150–190 nm, narrow size distribution (polydispersity index <0.2), and zeta potential of around +25 mV. Aqueous suspensions of zein nanoparticles were stable for at least 30 days when stored at 4°C. Maresin-1 alone and maresin-1-loaded zein nanoparticles presented low cytotoxicity to human macrophages. Moreover, its effect did not alter cell morphology. The obtained results showed that the nanoparticles induced an increase in macrophage polarization towards an M2-like phenotype, when compared with zein nanoparticles without Mar1. In addition, the higher concentration of Mar1 loaded into the nanoparticles led to a higher number of M2-like macrophages out of all experimental conditions.

Conclusions

Maresin-1 loaded zein nanoparticles were successfully produced. The newly developed nanoparticles were not cytotoxic to human macrophages. In addition, normal cell morphology was maintained up to 7 days of culture. The developed nanoparticles, in particular the ones with a higher concentration of Mar1, were able to modulate macrophage polarization towards an M2 anti-inflammatory and pro-regenerative phenotype. As such, the herein described nanoparticles will be able to promote pro-regenerative microenvironments.

Acknowledgments

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FABRICATION AND CHARACTERIZATION OF FUNCTIONALIZED PVA BUCCAL FILM FOR DRUG DELIVERY OF BOSWELLIA SERATTA

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Introduction

Biopolymers have played a significant role in human history; nevertheless, synthetic materials such as polyvinyl alcohol (PVA) have good mechanical, chemical, and physical properties [1]**.** These properties are partly contingent on the chemical features of the polymer, such as their chemical binding nature, types of functional groups, and how the functional groups are attached to the polymer chain[2]. Functionalization of PVA through the addition of chemical organic or organometallic groups has been incorporated or covalently anchored onto polymer matrices, their branches or arms, or their terminals to confer the desired properties, and their applications include sensors, biosensors, actuators, drug delivery, sutureless wound closure, and photovoltaics [3]. The present work focuses on modified PVA with an anionic and cationic moiety. The sodium carboxymethylcellulose (SCMC) and potassium chloride (KCl) were used as anionic and cationic moiety which were incorporated with PVA materials and evaluated the drug delivery of Boswellia seratta. FTIR and XRD were utilized to characterize the formulated films. Various techniques, such as swelling studies, surface pH, wetting studies, and mucoadhesion strength, were used to assess the physicochemical properties and mucoadhesion characteristics. The in-vitro and ex-vivo profiles of each film's drug delivery were investigated. These films were optimized with respect to their swelling and drug delivery properties.

Materials and Methods

Polyvinyl alcohol (PVA) (Fisher Scientific) with 35-50 cp viscosity and degree of hydrolysis 85-89%. Sodium carboxy methylcellulose (SCMC), Phosphate buffered saline (PBS) pH 6.8, Potassium chloride (KCl), were purchased from Sigma-Aldrich and other chemicals were purchased and used in this preparation process were of analytical grade.

Five different types of buccal films with the drug Boswellia serrata were prepared by using the solvent casting process with PVA and two different dopants, sodium carboxymethylcellulose (SCMC) as an anionic moiety and potassium chloride (KCl) as a cationic moiety. In each formulation, PVA, drug, and plasticizer were maintained as constants.

Results and Discussion

As the contraction characteristics of the SCMC material have a significant impact on the length of the film, the flatness of the films was evaluated between 95% and 98%. As a result of the mutual repulsion of the negatively charged carboxylate groups of SCMC, a PVA buccal film with a high concentration of SCMC exhibited high swelling properties. Studies of FTIR and XRD characterization revealed the formation of intermolecular hydrogen bonds between SCMC and –OH groups in PVA. This hydrogen bonding reduces the PVA molecule's entanglement. According to in-vitro and ex-vivo permeability experiments, the drug release profile during a 60-minute period follows the pattern A1<A2<A3<A4<A5**.**In the case of PVA/KCL buccal film, Due to the incorporation of KCl into the polymeric matrix, it was observed that the degree of crystallinity decreased. The ex vivo and in vitro drug delivery profiles of 'B' followed the pattern B1<B2<B3<B4<B5. According to the FTIR analysis, as the concentration of KCL in the PVA matrix rises, the band with a frequency range of 3095 cm^{-1} –3547 cm^{-1} becomes broader due to the OH stretch. Due to the formation of cross-links between the K+ cations and the oxygen atoms of the carbonyl groups, the observed peaks at 1726 cm^{-1} and 925 cm^{-1} indicate that the position of the peaks has not altered, but their intensities have decreased**.**

Conclusions

PVA buccal films with anionic and cationic moiety have been prepared by solution casting method. The characterization results shows that the addition of anionic and cationic moiety reduce the crystanility in XRD and shift the peak range in FTIR. In-vitro and In-vivo drug delivery characteristics of PVA/SCMC and PVA/KCl films. reveals that the addition of anionic content (3(w/v) %) in the PVA matrix found moderate drug-releasing characteristics and optimized ones. The addition of KCl (w/v) to 1% (w/v) PVA matrix as a cationic moiety shows moderate drug releasing characteristics. Based on the evaluation of these two types of films, it was concluded that the cationic moiety (KCl) film gives satisfactory results for drug delivery applications.

Acknowledgments

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BIO-SILICA AND BIO-POLYPHOSPHATE: APPLICATIONS IN BIOMEDICINE (BONE FORMATION)

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Introduction

The first mineralized metazoan skeletons consist of bio-silica and appear in the siliceous sponges, a taxon that has successfully survived the last 700 million years [1]. The calcium-based animal skeletons, first as crystalline calcite or aragonite ($CaCO_3$) in corals and subsequently as metabolically more active calcium phosphate, as crystalline calcium hydroxyapatite [HA] ($3Ca_3[PO_4]_2Ca[OH]_2$) developed later in vertebrates, about 560 million years ago [2]. The ancient (Proterozoic) ocean was rich in silicate, moderate in phosphate and very poor in calcium [2]. This composition of the marine environment was surely vital for the selection of silicate as the inorganic component in the first hard skeleton (spicules) of the siliceous sponges. In recent years, it has been disclosed that all metazoan taxa, including the sponges, originated from a single ancestor (the Urmetazoa) that already possessed the common genetic toolkits required for the development and morphogenesis of present-day animals (reviewed in [1,2]). Such a common genetic ground implies that the organization of the body plan, and in turn the skeletons of higher metazoans, follows a principle of a common architecture (reviewed in [1,2]). Furthermore, this suggestion implies that the silicate-based skeletons of sponges served as blueprint for the development of calcium-based skeletons. This hypothesis has been impressively supported by the findings of Carlisle [2] and Schwarz and Milne [2] who showed that during bone formation a close metabolic relationship between silicate and calcium deposition exists in birds and mammals. In following work, the importance of silicate for bone formation was substantiated [reviewed in [1,2]. Then we disclosed further a second inorganic bio-polymer, bio-polyphosphate, that has recently gained importance in the context of morphogenetic potential during bone formation [3-5].

Materials and Methods

The preparation of the bio-silica [6] and polyphosphate [3-5] samples by traditional method and bio-3D-printing [7] for *in vitro*, as well as the animal test in specific have been described before [8,9].

Results and Discussion

It has been demonstrated that bio-silica causes *in vitro* a differential effect on the expression of the genes OPG and RANKL, encoding two mediators that control the tuned interaction of the anabolic (osteoblasts) and catabolic (osteoclasts) pathways in human bone cells. Since bio-silica [6] and bio-polyP [3-5] also induce the expression of the key mediator BMP2 which directs the differentiation of bone-forming progenitor cells to mature osteoblasts and in parallel inhibits the function of osteoclasts, they are promising candidates for treatment of osteoporosis and bone diseases [3-7].

Conclusions

The two natural inorganic polymers, bio-silica and bio-polyP are both formed enzymatically, either in sponges or in microorganisms. There, both biopolymers act as skeletal elements, in siliceous sponges as the mineral matrix of the siliceous spicules (reviewed in [1, 2]) or as reinforcing support in the cell walls of microorganisms. Both polymers are biocompatible, a finding that is comprehensively proven for bio-silica [6]. The modes of action of bio-silica [1,2] and bio-polyP [10] as demonstrated in *in vitro* and *in vivo* experiments, qualify both polymers to be a potential beneficial biomaterial for bone regeneration. Recent clinical studies even in human are very encouraging [to be published].

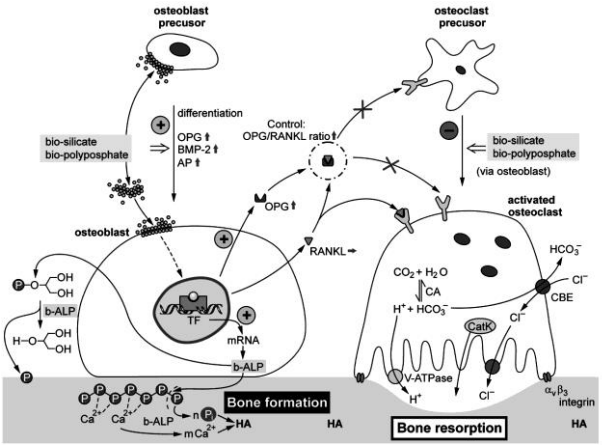

FIG. 1. Schematic outline of the tuned interaction of the cytokine/receptor triad RANKL-RANK-OPG and BMP2 that crucially controls the terminal differentiation steps of the osteoblasts and osteoclasts, in response to bio-silica and bio-polyphosphate.

Acknowledgments

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IMPACT OF AMINO GROUP DENSITY OF AMINE-BASED POLYMER COATINGS ON CELL RESPONSE

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Introduction

Demographic change in industrialized countries have resulted in an increased incidence of osteoporosis [1]. Integrating implants in the bone can be improved physically, but mainly by chemical modifications of the material surface. The initial step in osseointegration is the adherence of osteoblasts [2]. Amine-based coatings are known to improve initial cell attachment, spreading, migration, and intracellular signalling [3,4]. This work aimed to investigate the influence of amino group density on the biological effects of amine-based coatings and to understand these impacts.

Materials and Methods

Covalent grafting of polymer-based nano-coatings on titanium (Ti-Ref) surfaces (University of Technology Chemnitz, Germany) with different amino group densities was prepared by INNOVENT e.V. Jena (Germany), e.g., trimethoxysilylpropyl modified poly(ethyleneimine) (TMS-PEI). For protein adsorption analysis, size-exclusion HPLC with an Agilent AdvanceBIO SEC 300A was used (λ 215 nm). Wettability (WCA; Drop Shape Analyzer DSA25, Krüss), surface charges (zeta potential; SurPASS™ system, Anton Paar), and X-ray photoelectron spectroscopy (XPS; AXIS Ultra DLD XPS, Kratos Analytical) characterized the surface coatings. Human MG-63 osteoblasts (ATCC® CRL-1427™) [5] were cultured in DMEM (Life Technologies) with 10% FCS (Biochrom FCS Superior, Merck) and 1% antibiotics. For cell spreading, MG-63s were stained with PKH-26 (Sigma-Aldrich) [6]. Cell areas were determined via a confocal laser scanning microscope (LSM 780, Carl Zeiss) and ImageJ (NIH). Differential gene expression was analyzed with Clariom™ S microarrays (Applied Biosystems).

Results and Discussion

We could show a correlation between amino group density and the spreading behavior of cells (FIG. 1) and identified TMS-PEI as a coating with the highest amino group density. Also, we could demonstrate a premature activation of proliferation and differentiation after MG-63s cultivation on Ti-TMS-PEI. Protein adsorption assays revealed an increased BSA adsorption on Ti-TMS-PEI compared to the Ti-Ref.The fact that differentiation on amine-based coatings is enhanced has already been shown [7], but no microarray data are available so far. The effects of TMS-PEI shown here could compensate somewhat for the age-related slowing of osteoblast proliferation [8] and thus promote osseointegration.

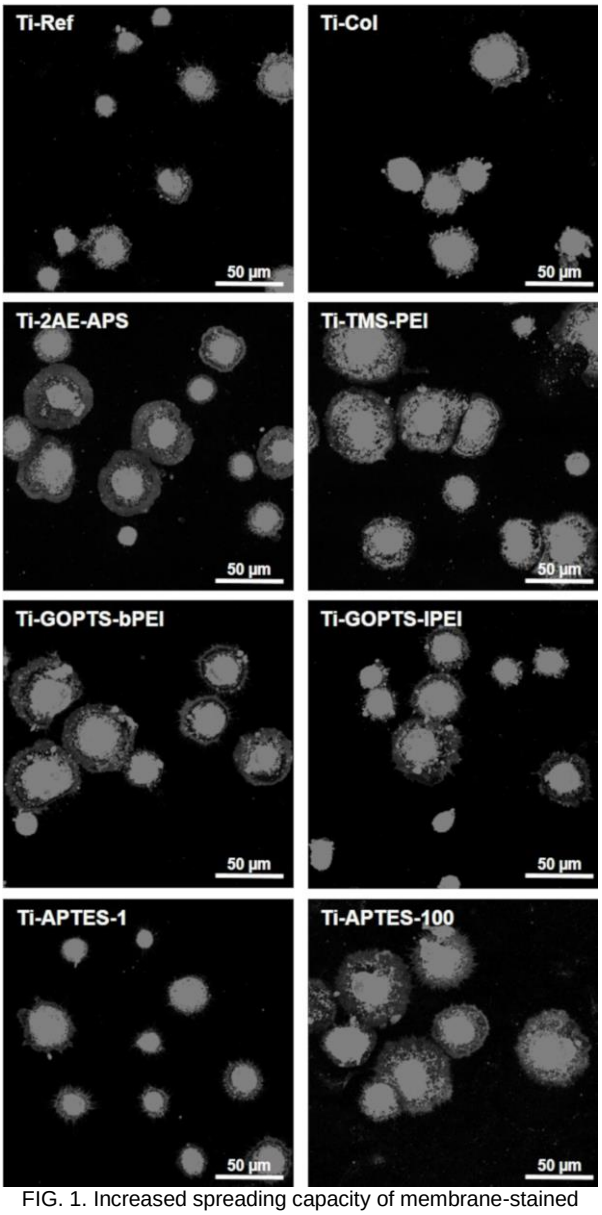

FIG. 1. Increased spreading capacity of membrane-stained (PKH-26) MG-63s after 30 min on amino coated titanium (Ti): N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (Ti-2AE-APS), trimethoxysilylpropyl modified poly(ethyleneimine) (Ti-TMS-PEI), *branched or linear poly(ethyleneimine) on (3-glycidyloxypropyl) trimethoxysilane (Ti-GOPTS-bPEI, Ti-GOPTS-lPEI),* *3*-aminopropyltriethoxysilane (Ti-APTES). Cell areas increased on all amino-rich nanolayers compared to reference titanium (Ti-Ref) and collagen I-coated titanium (Ti-COL) as controls.

Conclusions

The amino group density affected the spreading behavior of cells.

Acknowledgments

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Fe/HA BIODEGRADABLE SCAFFOLDS FOR THE NEEDS OF MODERN ORTHOPAEDICS

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Introduction

An important aspect related to the design of biodegradable implants is to increase their biointegration into bone tissue. Surface modification of orthopaedic implants is one of the effective strategies to accelerate bone healing in the early implantation period, and this is of great importance in relation to the use of temporary implants [1]. Among the various methods, coating implants with a layer of hydroxyapatite (HAp) is one of the most commonly used substances due to its excellent biocompatibility and osteoconductive ability [2]. Temporary implants should degrade at the same time as the formation of new bone, and after the regeneration process there would be no unnecessary elements left that could cause inflammation. An open-cell material with a pore size corresponding to cortical bone would allow blood vessels to penetrate deep into the implant and bone-forming cells. In addition, surface modification of temporary implants would be appropriate to obtain a highly biocompatible layer with a well-defined structure and nanoarchitecture. The hydroxyapatite layer on the scaffold should also have an impact for the degradation time.

Materials and Methods

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); FIG. 1 presents the scheme of the process.

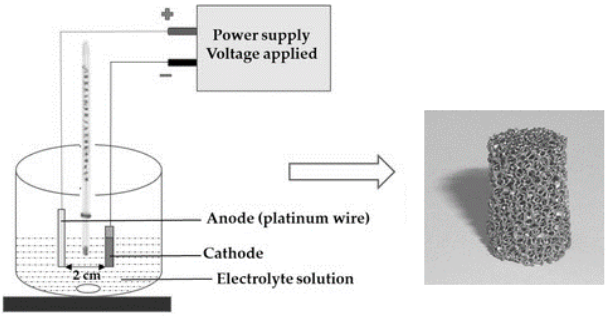

FIG. 1. The scheme of the production procedure.

The produced samples were morphologically characterised using a Quanta scanning electron microscope with field emission. Phase homogeneity was confirmed using X-ray diffraction. 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. In addition, nanomechanical tests (Nanointender Alemnis) such as nanohardness and reduced Young's modulus were carried out.

Results and Discussion

Layers of HY produced on iron 3D scaffolds differ in morphology and Ca:P ratio. FIG. 2 presents SEM images of HA layers obtained on Fe-scaffolds at three current intensities (0.5mA, 1.0mA and 1.5mA), as well as EDS spectra of obtained samples.

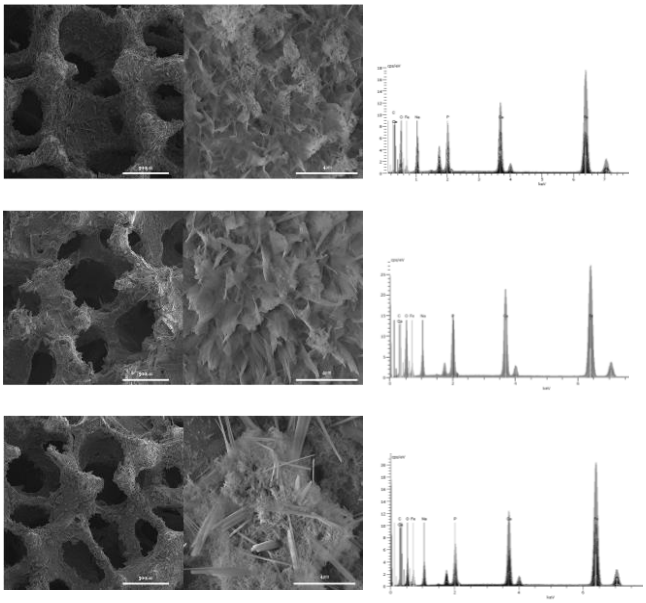

FIG. 2. SEM images of images of HA layers obtained on Fe-scaffolds at three current intensities: 0.5mA, 1.0mA and 1.5mA, and EDS spectra of obtained samples.

On the base of EDS spectra it was possible to estimate the Ca:P ratio and the values are as follows; 1:32, 1:57 and 1:69 for HA layers obtained at 0.5mA, 1.0mA and 1.5mA, adequately.
Corrosion behaviour of studied samples, in the form of polarization curves, determined using immersion and potentiodynamic polarization methods, are presented on the FIG. 3.

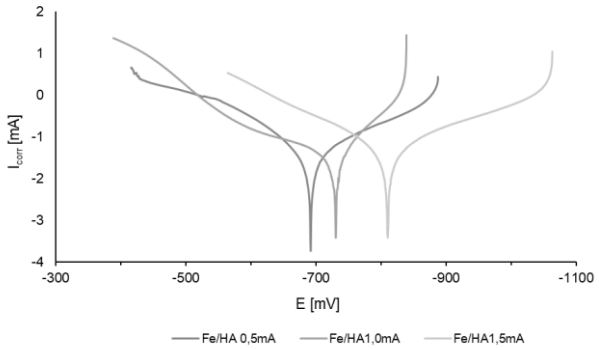

FIG. 3. Polarization curves of studied samples Fe/HA.

Conclusions

The process of optimizing the production of hydroxyapatite on the surface of a 3D iron scaffold was successfully carried out. The presence of a layer of hydroxyapatite on the surface significantly influenced corrosion properties of the whole system Fe/HA, accelerating it.

Acknowledgments

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TERPINEN-4-OL/CHITOSAN COATINGS DEVELOPED BY ELECTROPHORETIC DEPOSITION: MICROSTRUCTURE AND SURFACE PROPERTIES

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Introduction

Electrophoretic deposition (EPD) as a method for obtaining therapeutic coatings for short-term implants is currently being widely studied [1]. One of the most promising matrices for such coatings is chitosan. It is a natural polymer obtained from chitin, which is the main component of crustacean shells as well as some types of fungi. Chitosan exhibits antimicrobial and antifungal properties and is neutral to the human organism [2]. Its properties can be enhanced by the introduction of other natural materials. Among the most promising are essential oils and their components [3]. Terpinen-4-ol is a primary component of tea tree essential oil (TTO) and an isomer of terpineol. In this work, terpinen-4-ol/chitosan coatings were developed by EPD on titanium substrates. The main focus was put on the investigation of EPD kinetics and mechanism, as well as on characterisation of coating microstructure, morphology, surface topography wettability and surface free energy (SFE).

Materials and Methods

The coatings were deposited on three types of commercially pure titanium Grade 1: as-delivered, etched in HF and HNO_3 solution, and ground with 600 grit sandpaper. The suspensions used for the EPD were obtained by mixing terpinen-4-ol with chitosan solution in distilled water and CH_3COOH as well as EtOH with a speed of 20000/min for 6 min. The EPD parameters were set to a voltage of 10 V and a time of 5 min. The microstructure and morphology were examined by scanning- and transmission electron microscopy (SEM, TEM) including STEM-HAADF. The adhesion of coatings to titanium substrates was investigated using the tape-test in accordance with ASTM D3359-B. Surface topography and roughness were analysed by optical profilometry. Data on wettability and SFE were obtained using a sessile drop method. To assess bactericidal properties, the surfaces were incubated with *S. aureus* ATCC 25923 for 4 hours in 1 ml of a bacterial suspension at a concentration of 10^6 CFU/ml in PBS. Then, the surfaces were rinsed 3 times in PBS and the dead bacteria were stained with propidium Iodide. The images were taken by fluorescence microscope and parameterized in the ImageJ 1.53e program.

Results and Discussion

It was observed that the substrate used had an influence on the EPD process. The highest values of the deposition current density were obtained for as-delivered titanium and the lowest values were obtained for etched titanium. The surface preparation of substrates had a low influence on the EPD kinetics. Zeta potential measurements and TEM analysis of suspensions used for EPD allowed a possible mechanism of coating deposition to be formulated. Cationic chitosan molecules were adsorbed on terpinen-4-ol (FIG. 1a) and both moved towards the cathode and deposited on it. The substrate preparation before EPD also had an impact on the adhesion strength of the coatings. It was noted that as-delivered titanium is a very poor choice for the deposition of coatings due to its lack of adhesion strength (adhesion class 0B). For the other substrates, the adhesion was classified as high (class 4B) or excellent (class 5B).
SEM analysis showed that the substrate used has a low impact on the microstructure and morphology of the coatings. For both etched and ground titanium, the obtained coatings were homogeneous with terpinen-4-ol droplets regularly scattered on the surface.
The surface topography and roughness analysis performed by optical profilometry showed that the coatings obtained on the etched substrates were more developed and rougher than those obtained on the grinded titanium. This observation can lead to the prediction that coatings on ground titanium will have microbicidal abilities higher than those obtained on etched titanium.
The coatings obtained on the etched substrates had lower wettability but higher SFE compared to the coatings deposited on the ground titanium. This led to the conclusion that for further investigation of electrochemical corrosion resistance and antimicrobial properties, only coatings obtained on ground titanium will be taken into consideration. Furthermore, the terpinen-4-ol/chitosan surface was more bactericidal against *S. aureus* than the uncoated surface (FIG. 1b).

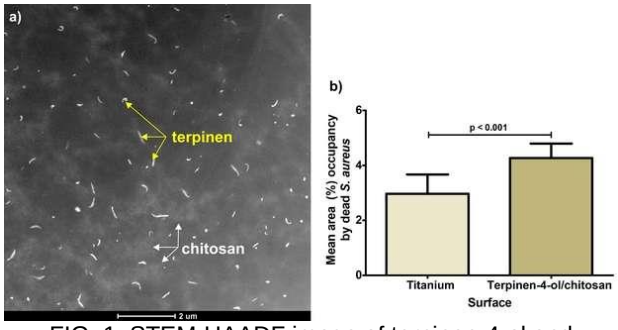

FIG. 1. STEM-HAADF image of terpinen-4-ol and chitosan in the suspension used for EPD (a) and mean surface area (%) occupied by dead S. aureus bacteria on the tested surfaces (b).

Conclusions

Natural chitosan coatings that incorporate terpinene-4-ol were successfully developed. Surface preparation of substrates had an impact on both the EPD of coatings and their properties. Despite similar adhesion strength, the coatings obtained on ground substrates can be preferable for further investigation due to surface roughness and surface properties, as well as demonstrated bactericidal activity against *S. aureus* in a short exposure time. Further optimization and characterization of the biomaterials are in progress.

Acknowledgments

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NOVEL POLY(ALKYLENE CITRATE)-BASED MATERIALS FOR VASCULAR TISSUE ENGINEERING

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Introduction

The blood vessel substitutes of small diameter (< 6 mm) still remain the holy grail of vascular tissue engineering. Materials suitable as scaffolds for vascular grafts must be well-biocompatible and non-toxic, non-thrombogenic and non-immunogenic, biodegradable within the adequate time window, must have mechanical properties matching those of natural tissues, and its production should be economically viable. It seems that our newly-developed poly(alkylene citrate)-based materials (PAC, FIG. 1A) fulfill each of these conditions. We tested two types of PAC: cPOC, i.e. cross-linked poly(1,8-octamethylene citrate) [1] and cPHC, i.e. cross-linked poly(1,6-hexamethylene citrate) [2].

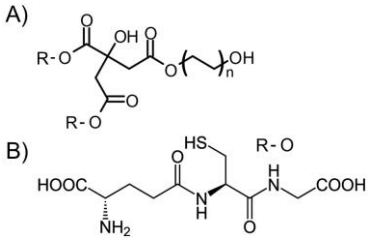

FIG. 1. A) General formula of PAC; B) Structural formula of glutathione

As oxidative stress is one of the major cardiovascular problems, an antioxidative action would be advantageous property of vascular scaffolds. For this reason, we modified PACs with glutathione (GSH), i.e. a low-molecular-weight thiol protective against oxidative stress (FIG. 1B), in four different concentrations (0, 0.4, 0.8 and 1.6 % w/w).

Materials and Methods

The efficacy of material cross-linking and its potential alteration by GSH were studied using solid-state nuclear magnetic resonance (ssNMR). For testing biocompatibility, i.e. the adhesion and proliferation of cells relevant for vascular tissue engineering, we used human adipose-derived stem cells (ASCs), normal human dermal fibroblasts (NHDFs), human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (SMCs). The latter three cell types are naturally present in human vascular tissues, whereas ASCs can be used for differentiation into all three types of vascular cells. Prior to cell seeding, the materials were extensively rinsed with cell culture media in order to remove non-cross-linked acidic groups. The cell proliferation rate was estimated using a resazurin metabolic assay. The addition of 500 μM menadione (an oxidative agent) was used for testing the capability of GSH-containing materials to protect cells against oxidative stress. Statistical analysis of the data was performed using a one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test.

Results and Discussion

In order to provide a pH-stable environment for cell growth, it is necessary to ensure good cross-linking of citric acid groups. The ssNMR spectra of cross-linked POC with or without GSH (FIG. 2) confirm that most of the materials are cross-linked well (by comparison of peaks height and area under the peaks corresponding to cross-linked and free acid and octamethylene groups). Insert in FIG. 2 compares signals from non-cross-linked acidic groups in POC with different GSH content. All three signals fit well together, therefore GSH addition does not have any influence on the extent of cross-linking.

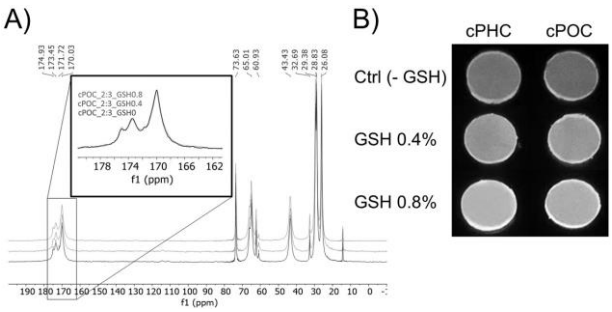

FIG. 2. A) Comparison of ssNMR spectra of cPOC modified with GSH (0%, 0.4% and 0.8%). Insert: Detail of frequency range corresponding to the signal of unreacted (non-cross-linked) carboxyl groups; B) Fluorescence dependent on GSH content.

However, one of the usable material properties is their fluorescence increasing with GSH content (FIG. 2B).
The cell-protective effect of GSH was confirmed using a resazurin metabolic assay (FIG. 3). The most pronounced anti-oxidative effect of GSH, simultaneously with a high cell sensitivity toward the oxidative stress was observed in ASCs. Relatively high sensitivity to oxidative stress was also noticed in NHDFs. However, the sensitivity of SMCs, and particularly HUVECs, was relatively low, probably due to their localization in the vascular wall frequently exposed to oxidative agents.

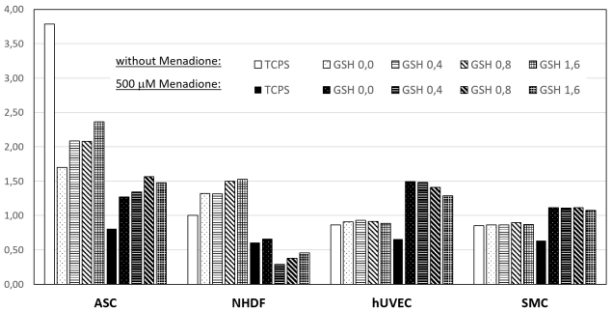

FIG. 3. Resazurin test: the influence of 500μM menadione on metabolic profile and survival of four cell types seeded on tissue culture polystyrene (TCPS) and cPHC (+/-GSH).

Conclusions

Our cPAC materials seem to be good candidates for the construction of tissue-engineered vascular grafts. Their advantage is also antioxidant action and fluorescence, which can be further used in *in vivo* experiments.

Acknowledgments

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MECHANICAL CIRCULATORY SUPPORT FOR CHILDREN - CONSTRUCTION AND MATERIAL CHALLENGES

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Introduction

The number of patients with heart failure has been steadily increasing in recent decades. In the United States, the number of patients has exceeded 5.7 million people, more than half of whom die within five years of diagnosis. In Poland, the number of patients with this disease is approx. 1.24 million, which is 3.2% of the population of our country. What is alarming, the number of children with heart failure and children qualified for heart transplantation is constantly increasing. In the United States, it is estimated that 12,000 to 35,000 patients under the age of 19 are affected by heart failure each year. The long waiting time for an organ and the fact that the anatomical and physiological features vary depending on the patient's age mean that the mortality rate in this group is high. 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. Pulsatile extracorporeal pumps are also the only alternative to heart transplantation for children. Currently, the 3rd generation fully implantable centrifugal blood pumps, most commonly used in adults, have very little use in children due to the limited space in the chest. For this reason, the Foundation of Cardiac Surgery Development (FRK) conducts R&D works aimed at developing the Polish family of extracorporeal paediatrics pumps for cardiac support and preparing its implementation in the clinics.

Materials and Methods

The implementation of a complex project goal requires solving many multidisciplinary challenges from the borderline of mechanical and material engineering as well as biological and medical sciences. In this work, we present examples of construction and material challenges that are of key importance in clinical practice, considering bio- and hemo-compatibility. The work will discuss the aspect of asymmetric construction of the blood chamber and valve system, but also material engineering issues including sliding coating in the membrane system and athrombogenic coatings in the flow domain. The project was defined as a response to an urgent medical need to increase the effectiveness of heart failure treatment for children by reducing mortality and provide patients with the necessary time for transplantation. Blood clot formation is one of the most common postoperative complications for patients who use blood-contacting medical devices. It is therefore very important to ensure the proper balance between design and functionality to ensure the required flow parameters while limiting shear stresses and eliminating areas of so-called shadows, characterized by blood stagnation, leading to embolization of the pump. As part of the current work, related to the application of surface engineering techniques, a modification of the sliding surface has been developed and optimized. The membrane system consists of two diaphragms working together: a blood diaphragm and a pneumatic working diaphragm. These elements are made of PU, and to ensure high wear resistance during long-term operation, it is necessary to use a sliding coating that reduces the friction coefficient. For this purpose, modifications were carried out using nanographene powder. Also, the internal surfaces of the device in contact with blood require the use of surface modifications ensuring high hemocompatibility in a dynamic flow system. Foreign surfaces placed in the human body do not possess thrombosis-resistant mechanisms. Instead, they activate a series of interconnected coagulation processes that include protein adsorption, platelet adhesion and activation, thrombin generation, and complement system activation. The initiation processes of thrombus formation in assistive heart devices may result in activation of the coagulation system, and ultimately embolization of the pump, preventing proper therapy and threatening the lives of patients. The most common cause of thrombosis is inadequate flow dynamics in the blood chamber, high shear stresses, and the material aspect due to the interaction of the artificial surfaces of the devices with the circulating blood. The overall incidence of thrombosis in VAD flow pumps is reported to be up to 30%. Importantly, the risk of pump thrombosis is the highest during 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 post-operative haemorrhage, prophylaxis of antithrombotic therapy is limited during this period. One of the solutions may therefore be the optimization of blood-contacting materials, considering new surface engineering techniques, and ensuring a high biosafety factor, including hemocompatibility. As part of the work, modifications were carried out using oligoproline and carbon-nitrided coatings.

Results and Discussion

The computer-aided analyses allowed to develop the series of extracorporeal heart support pumps RELIGA HEART PED in sizes: 12, 20, 30 and 45 ml. Due to the complex reactions in the body during cardiac support, each chamber size was designed and optimized individually. The studies were then validated on a physical model of the pump during laboratory tests including hemocompatibility assessment. In addition, the devices were optimized in terms of valve system. The single-disc mechanical valve was replaced with a single-leaflet PU valve developed by FRK. The construction of the mechanical outflow valve was equipped with a stabilizing cover that reduces stress and vibration during its operation. The surface modification within the membrane system was carried out using a 6-axis Mitsubishi robot with the activation by cold plasma treatment and nanographene spraying. In the case of athrombogenic layers, oligoproline-based coatings showed strong prevention in the adsorption of serum proteins and the adhesion of fibroblasts. Therefore, these coatings have the potential to be used as a novel type of antithrombotic coating.

Conclusions

The results present the progress of work on the Polish family of paediatric extracorporeal pumps RELIGA HEART PED for cardiac support and selected challenges from the borderline of medical and engineering sciences.

Acknowledgments

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INSIGHT INTO INFLUENCE OF STERYLIZATION PARAMETERS ON POLYPYRROLE COATING

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Introduction

Sterilization is an important step of manufacturing of implants or medical devices aimed at prevention of the spread of infection [1]. Each material with perspective application in the field of bioengineering must be validated in terms of its stability after sterilisation procedure. Among different methods steam and dry heat sterilization, ionising radiation, ethylene oxide (EO) sterilization and plasma discharges are most commonly used. The choice of the method is dictated by its efficiency, material stability and its proneness to degradation processes [2]. In the work we decided to determine the effect of sterilisation process conditions on the properties of polypyrrole (pPy). Material deposited by electrochemical techniques in the presence of surface active compound namely sodium dodecyl sulphate (SDS) was tested. It is a promising material for fabrication of coating layer on neuroactive implant devices.

Materials and Methods

The coatings were deposited with electrochemical methods either cyclic voltammetry (CV) or chronoamperometry (CR) to detect the influence of the experimental condition like potential protocol or rate of polarisation on the stability of the material. All synthesis were run in air conditioned room at stable temperature. The coatings produced on the flat medical steel surface were either steam or UV sterilised. Steam sterilisation was carried out in a ZEALWAY GR60DA autoclave at a pressure of 0.1 MPa. The sterilisation procedure involved 2 sets namely 120°C for 15 min and 134°C for 3 min. Sterilisation by UV radiation was carried out under a 36 W lamp as radiation source (λ=365 nm). Sterilisation times set as 15 and 30 min respectively. For both procedures samples were packed in special foil-paper sleeves for sterilisation (MEDAL). After sterilisation act the materials were characterised by recording CV curves to estimate its electroactivity and SEM imaging to trace the morphology changes.

Results and Discussion

Application of chosen parameters in the electropolymerisation procedure realised with cyclic voltammetry led to deposition of films characterised with varying thickness, electrochemical stability. The properties of the films were decisively influenced by the value of the applied potential and the number of scanning cycles.

Investigations of the electrochemical response during doping and de-doping cycles in the electrolyte solution using the CV method showed a stable increase in the charge density recorded during the anodic cycle (FIG. 1). This was linked to conformational changes occurring in the material immersed in pure electrolyte environment. The application of a steam sterilisation at 120°C for 15 min did not significantly affect the electrochemical stability of the material, nor the conductivity and molecular structure of the polymer films. However, increasing the temperature to 134°C for a reduced exposure time (3 min) led to degradation of the polymer matrix. It was visible in terms of electrical activity, reduced stability as well as electrical conductivity of the studied materials.

A significant effect of ultraviolet radiation was found, which led to a decrease in the adhesion of the polymer layers to the electrode surface, resulting in mechanical damage. The morphology of pure polypyrrole coating is characterised by star – shaped structures (five-pointed or four-pointed structures), triangular or globular structures are also visible. They can be seen to vary in size, as well as forming agglomerated systems. The obtained coatings wrinkled, corrugated, which is due to the presence of SDS micelles in the synthesis process - the chain formation process took place in the space around the micelles formed in the vicinity of the electrode surface. For coatings subjected to the steam sterilisation process, the observed morphology does not change, retaining distinct star-shaped structures (FIG. 2). For samples with different temperatures of the sterilisation process (120°C vs. 134°C), greater undulations are visible, indicating a tendency towards agglomeration when the temperature is increased. This leads to a reduction in charge dissipation capacity, resulting in lower conductivity. In the case of intact coatings sterilised by UV doses, no significant differences in structure were observed, retaining the globular morphology typical of polypyrrole.

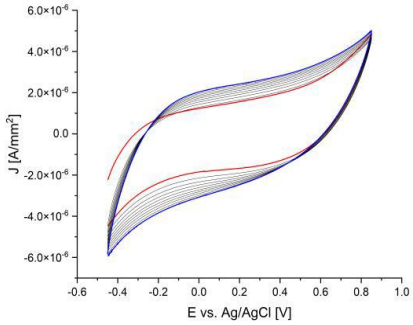

FIG. 1. CV curves of pPy film after steam sterilization (condition: 120°C, 15 min).

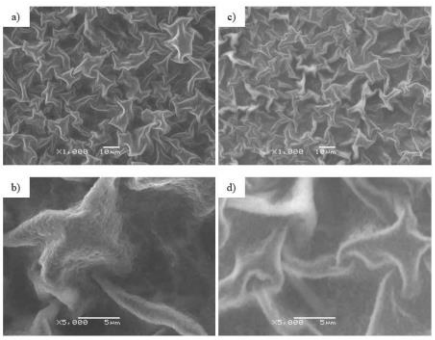

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FIG. 2. SEM images of films after steam sterilization in temperature a), b) 120°C and c), d) 134°C.

Conclusions

Steam sterilisation at 120°C for 15 min does not significantly affect the electrochemical stability, conductivity and molecular structure of studies polymer electroactive films. However, rising the temperature up to 134°C for 3 min can contribute to decrease in electrical activity with reduced stability. Application of UV radiation deteriorates the adhesion of the polymer layers to the electrode surface, which contributes to mechanical damage.

Acknowledgments

The work was supported by Institute own funding.

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ANTIBACTERIAL ACTIVITY AND CYTOTOXICITY OF BIORESORBABLE POLYESTERAMINES SYNTHESISED FOR USE IN BIOMEDICINE AND COSMETOLOGY

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Introduction

Biomaterials used in tissue reconstruction or during surgical procedures are exposed to pathogen attacks. After implantation, an infection may result in inflammation in the tissue due to infection and the growth of staphylococci or fungi. Post-implantation bacterial infections are a significant problem in modern surgery. Of the clinical complications associated with the implantation procedure, infections related to the biomaterial are assigned the leading role. Therefore, it is essential that the implant or scaffold intended for cell culture is protected against bacterial activity until the tissue is vascularized. For many biomedical applications, a bioresorbable material with bactericidal properties, better wet by water and presented better cell adhesion than currently commonly used aliphatic copolyesters is sought. Due to the presence of chemically active functional groups, it should possess also be easy to modify by attaching proteins, polypeptides or other biologically active compounds. These requirements are largely met by aliphatic polyesteramines, obtained by enzymatic polymerization of ROP of pentadecalactone in the presence of diethanolamine [1]. In our research, similar triblock polyesteramines were obtained using a different method, which allowed for obtaining a larger range of polymers with different properties, additionally containing pendant hydroxyl groups.

Materials and Methods

The first step in the synthesis of high-molecular polyesteramines with pendant hydroxyl groups was the polycondensation of tartaric acid esters and secondary amines terminated with hydroxyl groups; diethanolamine (DEA) and N, N'-bis(2-hydroxyethyl) ethylenediamine (HEDEA). The polyesteramines obtained in this way were used in the next stage of the synthesis as ROP macroinitiators for the copolymerization of L-lactide and glycolide. However, to be able to carry out the planned reactions, it was previously necessary to block the hydroxyl groups in the tartaric acid ester as well as the secondary amino groups in the hydroxylamines. The group protecting conditions were chosen so that during the planned syntheses, the blocking compounds were stable and non-reactive during the polymerizations carried out, and at the same time that the subsequent deactivation of functional groups in the final polymer could be carried out in a relatively simple and effective manner [2]. By selecting different macroinitiators, the copolymerization of L-lactide with glycolide (molar ratio as; 85:15) resulted in a series of copolymers with different compositions and chain structures. The obtained copolymers were subjected to cytotoxicity tests according to the ISO 10993-5 standard. The research was carried out using the human fibroblast line WI-38 (CCL-75) and keratinocytes (HaCaT). At the same time, the antibacterial and antifungal activity of the synthesized polymers was assessed using the broth dilution method, following the recommendations of the Clinical and Laboratory Standards Institute. The following strains were selected for the study: Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, and fungi: Aspergillus brasiliensis and yeast: Candida albicans.

Results and Discussion

High molecular terpolymers with a composition close to that assumed theoretically were synthesized. NMR studies showed that in the obtained terpolymer, the block formed by chain units derived from the macroinitiator was not terminated with OH groups, so they did not form chain ends. The molecular weight distribution on GPC chromatograms was monomodal and there was only one glass transition temperature in the DSC thermograms. All this proves that block terpolymers, not a mixture of two polymers were obtained during the copolymerization. After the deprotection of hydroxyl groups and secondary amino groups, the final terpolymer obtained was characterized by high hydrophilicity. After the deprotection of hydroxyl groups and secondary amino groups, the final terpolymer obtained was characterized by high hydrophilicity. Depending on the length of the hydrophilic block and composition of the terpolymer, the value of the water contact angle ranged from 50° to 34°. The obtained results of cytocompatibility studies did not show any toxic effect on the examined cells of fibroblasts and keratinocytes for most of the terpolymers. The obtained polymers showed different antibacterial activity depending on the type of bacteria, as well as on the structure of the polymer itself. All obtained polyesteramines exhibit bacteriostatic activity. However, they did not show antifungal activity against Aspergillus brasiliensis and Candida albicans strains. Poly(L-lactic-co-glycolide)-bloc-poly(diethanolamine tartrate)-bloc-poly(L-lactide-co glycolide) terpolymer with 20 mol.% hydrophilic amine block had an especially strong antibacterial effect. At a polymer concentration of 1 mg/ml within 24 hours, it was noted; a decrease of S. aureus and E. coli bacteria below 0.1%, Pseudomonas to about 1% of the amount measured in the control sample.

Conclusions

The manufactured polymeric materials seem to be particularly interesting as a material for use in tissue engineering. It also seems valuable to use the amphiphilic properties of the described materials in the creation of drug micro- and nano-carriers. These polymers in this form can be used as a component of creams or ointments, which is a carrier of biologically active compounds in cosmetics or dermatology. They will be involved in the gradual release of these compounds into the skin while providing protection against bacterial contamination.

Acknowledgments

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INVESTIGATING THE BIOACTIVITY OF COMPOSITE FILMS INCORPORATING POLYMER ENRICHED WITH TITANIUM(IV)-OXO COMPLEXES

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Introduction

The results of extensive research on modern technologies draw attention to the wide utilization of titanium dioxide-based materials in various aspects of our daily lives. The bandgap of TiO_2, with values of 2.9-3.2 eV for rutile and anatase, respectively, significantly affects the activity of these materials in the ultraviolet range, which limits their broader application. Moreover, determining these materials' precise structures, atomic positions, and connectivity remains challenging. In addition to their photocatalytic properties, TiO_2-based materials are studied for their bioactivity and potential application as antimicrobial and anti-inflammatory agents. The limitations arising from the absorption of UV radiation by TiO_2-based materials have led to research focused on modifying them to shift their catalytic properties and biological activity toward visible light. Simultaneously, there is a significant emphasis on developing materials that exhibit appropriate antimicrobial activity while ensuring low cytotoxicity and non-carcinogenic properties. To overcome the limitations of TiO_2-based materials, attention has been directed towards multinuclear titanium(IV)-oxo complexes (TOCs). The incorporation of organic ligands, which stabilize the $\{Ti_aO_b\}$ core structures of the oxo complexes, allows for adjusting the energy gap and facilitating the shift of the photocatalytic activity of these compounds towards visible light. The results of our previous research focused on TOCs stabilized with 9-fluorencarboxyl ligands on various core structures $\{Ti_aO_b\}$ (a = 3, 4, 6, b = 1, 2, 4, 6) revealed that these compounds exhibit photocatalytic activity in both the UV and visible range, following the sequence: $\{Ti_6O_6\}$ > $\{Ti_4O_2\}$ > $\{Ti_3O\}$ > $\{Ti_6O_4\}$ [1]. Additionally, these complexes showed antibacterial activity, with strong effectiveness against Staphylococcus aureus but weaker effectiveness against Escherichia coli [1]. It should be noted that the antimicrobial activity of TOCs remains relatively understudied. One significant drawback is their low solubility in water solutions and sensitivity to hydrolysis processes. The latter effect, demonstrated by the tetranuclear Ti(IV)-oxo complex stabilized by triclosan, can transport triclosan as an antibacterial agent, which will subsequently be released through hydrolysis processes [2]. Meanwhile, there is a search for effective agents with microbicidal properties that can be used, for example, in producing coatings or hydrogels. These agents should efficiently eliminate microorganisms when exposed to visible light and maintain their activity for an extended duration.

Materials and Methods

Synthesis of TOCs: The primary reagents for synthesizing (**1**) are titanium(IV) isopropoxide and 9-hydroxy-9-fluorenecarboxylic acid. The reaction is conducted in a glovebox at room temperature under an argon atmosphere. A molar ratio of 4:1 $Ti(^iOPr)_4$/carboxylic acid and a solvent mixture of THF/PriOH (1:1) are used. Synthesis (**2**) involves introducing propionic acid into the aforementioned mixture. Complex (**3**) is synthesized under the same conditions as (**1**), using mandelic acid. To protect TOCs against potential hydrolysis processes, biological activity studies of oxo complexes (**1**)-(**3**) are conducted for composite systems created by dispersing TOCs (approximately 2-20 wt.%) in a polymer matrix. The manufactured samples are characterized spectroscopically, and their physicochemical and mechanical properties, wetting, free surface energy, and ability to generate reactive oxygen species (ROS) are examined. The biological properties research of the produced composites allows for determining their antimicrobial activity, cytotoxicity, and anticancer properties.

Results and Discussion

In the context of our research, we investigated a new group of Ti(IV)-oxo complexes formed by the reaction of Ti(IV) isopropoxide with α-hydroxy-carboxylic acids, namely compounds (**1**) and (**3**). Adding these compounds to the reaction mixture, which can coordinate with Ti(IV) through carboxyl and hydroxyl groups, resulted in the formation of complexes with distinct physicochemical and biological properties compared to previously studied carboxylic acid-stabilized complexes [1]. To enhance the antimicrobial properties, we introduced propionic carboxylate ligands into the structure of (**1**), leading to the formation of compound crystals (**2**). Microbiological tests have unequivocally demonstrated the antimicrobial properties of composites enriched with (**2**) and (**3**). This is substantiated by the data presented in TABLE 1. The aforementioned systems exhibited no cytotoxicity or tumorigenic properties.

TABLE 1. Antimicrobial activity of PMMA + TOCs (TOCs = (**1**), (**2**), and (**3**)).

No.	Composite sample	Microorganisms				
		E. coli ATCC 8739	*E. coli* ATCC 25922	*S. aureus* ATCC 6538	*S. aureus* ATCC 25923	*C. albicans* ATCC 10231
1	PMMA	none	none	none	none	none
2	PMMA + (**1**) 10 wt.%	0.26	0.22	0.36	0.30	0.06
3	PMMA + (**2**) 10 wt.%	5.55	5.34	5.00	5.66	4.01
4	PMMA+ (**3**) 10 wt.%	4.90	2.90	4.70	5.10	0.80

Conclusions

The results of our research demonstrate that TOCs, which are stabilized using α-hydroxy-carboxylic ligands, exhibit improved structural stability, decreased vulnerability to hydrolysis processes, and a shift in their absorption maximum towards visible light. The antimicrobial activity of the TOCs is directly influenced by the functionalized carboxylate ligand chosen to stabilize the $\{Ti_aO_b\}$ core, as exemplified by complexes (**1**) and (**3**). Through our endeavors, we have successfully developed composite coatings, namely PMMA+(**2**) and PMMA+(**3**), that possess both potent antimicrobial properties and do not display cytotoxic effects.

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NON-INVASIVE POROSITY MEASUREMENT OF COMMONLY USED BIOMATERIALS BY NUCLEAR MAGNETIC RESONANCE

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Introduction

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 can be 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.

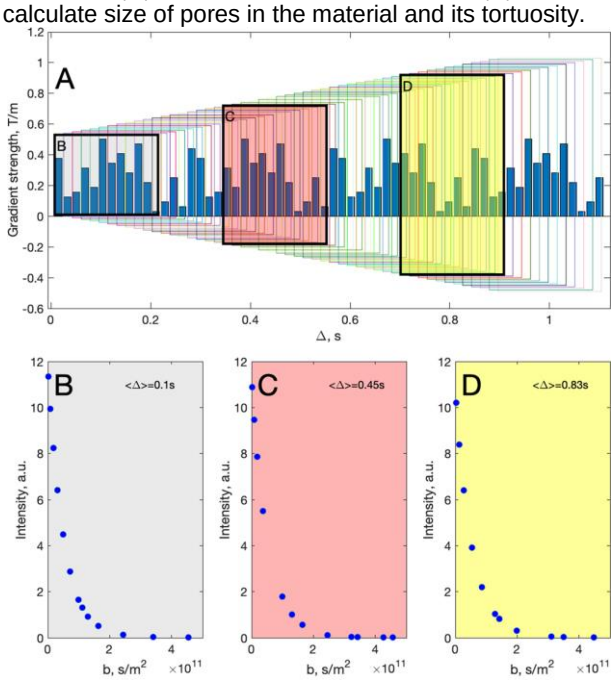

FIG. 1. TR-restricted diffusion experiment. (A) Repeating random gradient strengths while the Δ value is linearly increased. The experiment is cut into overlapping frames that are used to calculate diffusion coefficients. (B–D) Examples of signal intensities at three different average times (Δ).

Results and Discussion

From measurements we obtained data of apparent diffusion coefficient dependence on Δ for each sample. We assumed spherical shape of the pores. The radius of the pores were gained from fitting eq1 to data and tortuosity from fitting eq2 to data. We measured eight samples, which differed in concentration of GELMA and its degree of substitution (DS), which informs about percentage of substituted NH_2 and OH groups. The radii of pores are in range of few μm and are decreasing with increasing GELMA concentration and DS. The tortuosity increases together with both parameters. In addition, we have proved the high compatibility and convergence of the obtained results with the measurements made by the SEM/TEM method and literature data.

Conclusions

We investigated the structure of GELMA hydrogel by restricted diffusion measurements. The speeding up of these measurements using TR method was successful. We calculated the pores radius and tortuosity of material and got their dependence on concentration of GELMA and DS. We proved that applied technique provide excelent convergence with SEM/TEM results and literature data. Therefore presented methodology is a powerful tool for fast and non-invasive measurment of porosity of any biopolymers including both materials and scafolds with and without cells.

Acknowledgments

TECHMATSTRATEG-III/0027/2019-00/National Centre for Research and Development

Conflicts of Interest

Michal Wszoła and Marta Klak is the co-founder of Polbionica Ltd.

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DEVELOPMENT OF A METHOD OF APPLYING A LAYER OF CHITOSAN + BERBERINE ON A PEO-MODIFIED SPINAL IMPLANT MADE OF TI6AL4V, PRODUCED BY SLM METHOD

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Introduction

The development of additive technologies enables the production of implants with improved functionality. Thanks to the use of highly porous implants with a precisely designed structure of macropores, it is possible to obtain an element that not only has mechanical properties better suited to the properties of bone tissue, but also improves its osseointegration [1].
In order to obtain even better therapeutic properties, it is possible to use additional bioactive coatings. The use of a biodegradable biopolymer makes it possible to change the surface properties of the implant [2, 3].
Due to the extensive macrostructure of the implant, it is characterized by a high ratio of surface area to real volume, which is the potential for aggregation of a larger volume of biopolymer with the active substance both outside the implant and in its central part. The extensive macrostructure also enables the extension of the release time of the active substance, especially from the central part of the implant. The features of the implant that make this possible also present challenges when applying the polymer layer.
The paper presents the results of research aimed at the developed method of applying a layer of chitosan with the addition of berberine as an active substance on the surface of a proprietary spinal implant subjected to PEO treatment. On the basis of spectrophotometric tests, a method of applying a layer of chitosan + berberine with the most favorable kinetics of active substance release was determined.

Materials and Methods

The research was carried out on a proprietary spinal implant intended for the treatment of discopathy in the C4-C5 section. The implant is characterized by a highly porous macrostructure (porosity >50%, pore diameter 600 um, diamond mesh). The implants were produced at EHTIC in Zabrze on a printer EOS M 100 from Ti6Al4V powder. After production, samples were subjected to thermal treatment at 850°C in a protective gas environment. The implants were cleaned in an ultrasonic washer according to the scheme: deionized water (DW), water with detergent, DW, acetone, DW. The PEO process was carried out using 0.5M $Ca(H_2PO_2)_2$ and pulse current with the following parameters: j=50mA/cm^2, U=250V. Then the samples were cleaned in an ultrasonic washer according to the scheme: DW, acetone, DW.
5 g of low molecular weight chitosan (ALDRICH) was added to 400 cm^3 of the 2% acetic acid. The solution was stirred with a magnetic stirrer at 25°C for 24h. To 50 dm^3 of a 1.25% chitosan solution, 0.14 g of berberine (chloride form, SIGMA) was added and left on a magnetic stirrer until complete dissolution.
The samples were divided into 7 groups. Samples from groups 1-6, 0.4 cm^3 of the prepared solution was deposited, making sure that the solution penetrated the inside of the implant and left to dry under normal conditions. Samples 1-5 were covered with the dip coating method, the speed of immersion and ascent: 0.7 mm/s for different times and number of dips:
1. single dip for 15 minutes;
2. 3 dips, 5 minutes each, 5 minutes between dips;
3. 3 dips, the first one for 15 minutes, the next: down-up
4. 15 dips, each down-up, 5 minutes between dips
5. 3 dips, 5 minutes each, drying 2 hours between dips.
Then: 6. immersion in 5 ml of solution for 24 hours
7. immension in 15 ml of solution for 15 min with ultrasound.
After applying the layer, the samples were placed in the same positions and left for 48 h to dry under normal conditions. The presence of berberine on the surface of the implant was verified using ultraviolet light.
To release berberine from the implant surface, samples were placed in vials filled with 3 ml PBS and incubated at 37°C. Samples were transferred to successive vials at increasing time intervals. For the obtained filtrates, the amount of berberine released from the sample was determined by spectrophotometry. The determined concentrations were converted into the total amount of berberine released into the environment.

Results and Discussion

Verification using UV light confirmed the presence of berberine both on the outer surface and in the shallow layers of pores. During the application of the solution, it easily penetrated the interior of the implant. The coated implants showed high wettability for PBS solutions. The release kinetics of the active substance was similar for all samples, but had different values (FIG. 1). The fastest decay of the secretion and the smallest total mass of berberine were determined for the sample covered with the use of an ultrasonic cleaner. For the remaining groups, berberine secretion was present until the end of the experiment.

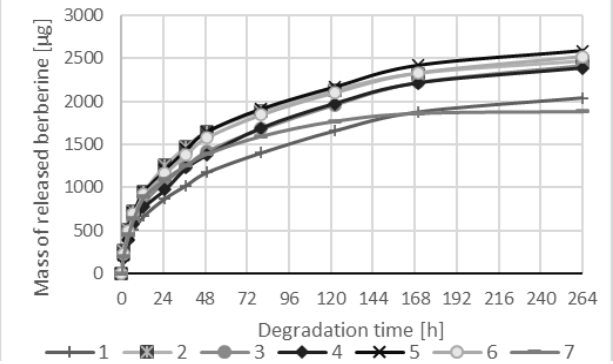

FIG. 1. Change in total mass of berberine released into the environment during incubation.

Conclusions

The method of applying the chitosan + berberine layer affects the volume of the active substance deposited on the implant. The release kinetics of the active substance decreases over time. The release of the active substance into the environment was maintained for more than 2 weeks. The largest volume of berberine was extracted from the implants covered with methods 5 and 6.

Acknowledgments

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ANTIBACTERIAL EFFICIENCY OF GOLD NANOPARTICLES/ GENTAMICIN HYBRID SYSTEM IN CHITOSAN LAYERS

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Introduction

The discovery of antibiotics was without a doubt revolutionary for the field of medicine, yet their indiscriminate use came under the scrutiny of scientist in recent decades due to increasing antibiotic resistance. It is estimated that by 2050, 10 million deaths worldwide will be related to antibiotic resistant infections [1]. While the search for new classes of antibiotics continues, researchers branch off to the alternatives, such as metallic nanoparticles [2], polyphenols [3] or antimicrobial peptides [4]. Furthermore, the variety of approaches can be combined to achieve additive or synergistic effects.

Among proposed solutions, gold nanoparticles (AuNPs) possess several advantages. Their biocompatible and chemically benign nature, combined with a wide choice of protecting ligands provide a good template for drug delivery systems. Described traits add to high scientific interest in conjugating AuNPs with antibiotics, cancer drugs or photosensitizers for photothermal/photodynamic therapy.

Presented study explores possibility of a combined approach in preventing bacterial colonization of biomaterials. It compares and quantifies antibacterial efficiency of the prepared chitosan layers that contain gold nanoparticles and gentamicin (AuNPs@GE) against Gram-positive and Gram-negative bacteria to antibiotic alone.

Materials and Methods

The AuNPs were prepared by coreduction of $HAuCl_4$ by GE and chitosan (CS). Briefly, 5.25 ml of 0.05 mM $HAuCl_4$ was added to varying concentration of GE sulphate in 21 ml of 1% (w/v) chitosan (medium molecular weight, 1202 ± 2 kDa, deacetylation degree 83 ± 5%) at 90°C, for varying amount of time. The size and morphology were estimated by UV-VIS spectroscopy, DLS and TEM. Chemical composition of samples were measured by ATR-IR spectroscopy and EDS.

The antibacterial activity was tested against Gram-negative *E. coli* and Gram-positive *B. Paramycoides* by both measuring inhibition zone on agar plates, as well as optical density (OD) at wavelength of 600 nm, after culturing bacteria for 24h in presence of tested material. Changes in bacteria morphology were observed under SEM, after fixating adherent bacteria by immersion in glutaraldehyde (2%) and dehydration in gradually increasing ethanol concentrations.

Results and Discussion

Synthesized AuNPs were spherical in shape and, on the average, 12 nm in diameter, well dispersed throughout chitosan matrix (FIG. 1a and 1b). The diameters obtained by TEM and DLS were both in agreement. The ATR-IR spectra confirmed presence of GE, unchanged by high temperature.

Zone of inhibition, as well as OD measurement both confirmed antibacterial activity of AuNPs@GE against Gram-positive and Gram-negative strains. The affected bacteria showed lower adhesion to samples, compared to chitosan alone, and disrupted cell walls (FIG. 1c). Additionally, AuNPs@GE proved to be significantly more effective at inhibiting both bacteria strains ($p>0.05$) than chitosan alone (CS) and chitosan layers loaded with just gentamicin of the same concentration (FIG. 1d).

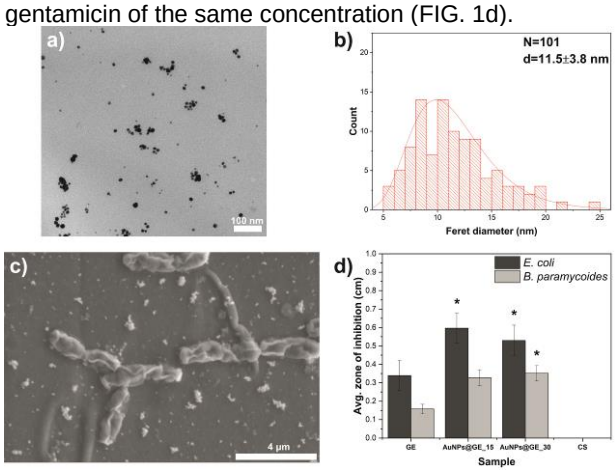

FIG. 1. a) TEM micrographs of AuNPs@GE after 15 min. of synthesis b) size distribution of synthesized nanoparticles c) Morphology of *B. paramycoides* on the surface of AuNPs in chitosan matrix d) zone of inhibition radius on agar plates containing *E. coli/B. paramycoides* and samples after 24h of growth. Asterisk marks statistical difference *vs.* GE ($p>0.05$).

Obtained results are in agreement with work of Payne *et al.*, where kanamycin conjugated AuNPs elicited increased antibacterial activity in comparison to antibiotic or nanoparticles alone [5]. In similar study, Sharma and Chaudhary ascribe enhanced antibacterial properties of GE conjugated AuNPs to increased reactive oxygen species (ROS) production and lipid peroxidation [6].

Conclusions

The study led to successful synthesis of stable, spherical AuNPs modified with gentamicin, around 12 nm in size. The nanoparticles were well dispersed in chitosan matrix and provided increased antibacterial efficiency against Gram-positive and Gram-negative bacteria strains, compared to the same concertation of antibiotic dispersed in chitosan.

Acknowledgments

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SOLVENT INFLUENCE ON GELATIN-ALGINATE HYDROGELS PROPERTIES

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Introduction

Hydrogels are three-dimensional materials that possess the ability to absorb a large amount of water or other solvents. However, depending on the purpose of the study, the solvent can differ and may cause different characteristics of obtained biomaterial. Water-based hydrogels have appropriate parameters for the basic studies of biomaterials [1]. But not only water can act as a solvent. A widely used basal medium supporting the growth of many mammalian cells, such as Dulbecco's Modified Eagle Medium (DMEM), is a perfect example of a more bio-based solvent. Gelatin and alginate salts as polar biopolymers dissolve quickly in water. On the other hand, alginate salts are converted into insoluble gels by cross-linking using divalent ions such as Ca^{2+} ions, which are present in very low concentrations in DMEM [2]. Thus the study aimed to test the characteristic parameters of gelatin_sodium alginate-based biomaterials in different solvents such as water and DMEM.

Materials and Methods

The examined basal biomaterial consisted of 6% gelatin and 2% sodium alginate- dissolved separately in water (G6_A2) and DMEM / F12K medium containing 10% FBS and a 1% mixture of antibiotics (identical to the 2D culture medium) (G6_A2_DMEM). Additionally, gels were enriched with two different cross-linking agents: 1% squaric acid (SQ) and 1% dialdehyde starch (DAS) based on the dry weight of the gelatin. The final step was the hydrogel immersion in a 1% calcium chloride solution for 10 min. Materials characterization was performed using the Shimadzu EZ testing machine (Shimadzu Corporation, Japan) for the mechanical properties. Viscosity tests were done using a Brookfield DV-1 Viscometer machine. Viscosity was measured at 40℃ at various 6, 12, 30, and 60 RPM speeds. The obtained results were processed using TRAPEZIUMX software and Microsoft Excel.

Results and Discussion

The tested gelatin_alginate hydrogels presented distinct properties depending on the solvent used for preparation. It was seen that starting gelatin_alginate solutions prepared in H_2O showed higher viscosity values than blends based on the culture medium. The viscosity of the base material decreases in the DMEM solution. However, the addition of a covalent cross-linking agent also affected the obtained viscosity values. A significant difference was visible for the SQ1 cross-linked material. Also mechanical properties differ depending on the solvent. Hydrogels prepared in DMEM showed higher values of Young's Modulus than those prepared in H_2O. Moreover, the G6_A2 hydrogel cross-linked by DAS presented increased Young's Modulus values with a slightly decreased percentage of elongation at the breaking point compared to the base material in H_2O.

Conclusions

To conclude, the change in the solvent used for the preparation of polymer solutions affected the viscosities of the obtained hydrogels. It is worth noting that the components in culture media increase the viscosity of gelatin_alginate solutions compared to water solutions. The mechanical properties tests confirmed the integrity and stability of the tested biomaterials. The stiffness of the material is significantly higher for DMEM-based hydrogels. That may suggest additional cross-linking of the material by the components present in DMEM, such as calcium ions. The presented results can be an important step for further characterization of gelatin_alginate hydrogels due to the possibility of the cell culture medium promising effects on tested material.

Acknowledgments

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A MEDICAL DEVICE ON THE MARKET - THE PATH FROM AN IDEA TO USE IN PATIENT

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Introduction
Medical devices play an essential role in saving lives by providing innovative solutions for diagnosing, preventing, monitoring, predicting, prognosticating, treating or mitigating disease.
There are over 500,000 on the EU market. types of medical devices and in vitro diagnostic medical devices. Examples of medical devices include: dressings, contact lenses, X-ray machines, pacemakers, breast implants, software applications and hip replacements.
European regulations ensure the safety and effectiveness of medical devices and facilitate patient access to these devices on the European market.
To keep up with technological progress, Medical Devices Regulation (EU) 2017/745 (MDR) replaced two medical device directives (Council Directive 90/385/EEC concerning active implantable medical devices (1990) and Council Directive 93/42/ EEC Medical Devices Regulation (1993)).
As the medical device sector experiences continuous and rapid development, ensuring a stable set of regulations is necessary to guarantee both safety and innovation.
Notified bodies ensure the safety and effectiveness of high-risk medical devices.
The notified body is responsible for verifying the compliance of the medical device with the general safety and performance requirements and participates in the procedure verifying this compliance, the properties of the medical device, the production process or the manufacturer's quality system. It has the authority to issue certificates of conformity, as well as to amend, impose restrictions, supplement, suspend, revalidate and withdraw certificates for all medical devices for which it has issued certificates within the scope of its notification. It carries out inspections and supervises manufacturers to whom it has issued certificates of conformity. The notified body obtains an identification number assigned by the European Commission and is included in the list of units - NANDO database.

Conclusions
CeCert is a dynamically developing certification and former notified body offering its services in certification: management systems, medical devices, training, supplier audits. In 2019, we were entered on the list of authorized ERCA partners in the field of audit training, and from 2020 we have been accredited by the Polish Center for Accreditation for the QMS PN-EN ISO 9001: 2015-10 and MDMS PN-EN ISO 13485: 2016-04 programs. In October 2021, the Minister of Health authorized CeCert in the field of in-vitro diagnostic medical devices and appointed it as a notified body. On January 3, 2022, we were assigned the number of a notified body - 2934. We are currently trying to obtain notification in the scope of Regulation 2017/745 regarding medical devices.
The presentation will focus on the definition of a medical device, the legal framework, risk classes of medical devices and important information regarding the creation of a medical device, as well as how conformity assessment by the Notified Body is carried out.

Acknowledgments
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RESEARCH OF CHITOSAN COATINGS DEPOSITED BY ELECTROPHORETIC DEPOSITION METHOD AT VARIOUS VOLTAGE AND TIME PARAMETERS

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Introduction

Chitosan is produced by removing a part of the acetyl group from chitin, often more than 60%. Chitin is a polymeric polysaccharide that is extracted from marine crustaceans. Due to its excellent biodegradability, biocompatibility, hypoallergenic, and biological activity (antibacterial, immunological, antitumor, and anti-inflammatory properties), chitosan has received considerable interest from researchers across the globe [1,2]. The aim of the research is to present the structure, wettability, and adhesion of the chitosan coating on the titanium alloy.

Materials and Methods

Chitosan coatings were deposited by using the electrophoretic deposition (EPD) method on the Ti13Zr13Nb at voltages of 20V, 25V, and 30V and times of 2min and 5min. The solution used for the deposition includes 0.104 g of high molecular weight chitosan, 100 ml of distilled water, and 1ml of 99.5% acetic acid. The solution was homogenized for 5 h. The tests were carried out using a scanning electron microscope (SEM), the wettability and adhesion of the coatings were also assessed.

Results and Discussion

Wettability test

The wettability of the coatings (n = 5 for samples) was evaluated by the falling drop method using water (1.8 µl) at room temperature (Optical Tensiometr Attension, Theta Lite, Biolin Scientific, Sweden). The computer program analyzed the contact angle (CA) for 10s. TABLE 1. presents the average value of CA after 10±0.07s.

TABLE 1. The value of the average contact angle.

Parameters	Average CA [°]
20V 2min	92.10 ± 2.69
20V 5min	78.22 ± 5.58
25V 2min	75.67 ± 9.80
25V 5min	83.42 ± 3.70
30V 2min	72.39 ±10.05
30V 5min	74.70 ± 2.75

Microscopic examination of the surface

A microscopic examination of the surface was carried out using a scanning electron microscope JSM-7800F. The images obtained are shown in FIGs. 1-3.

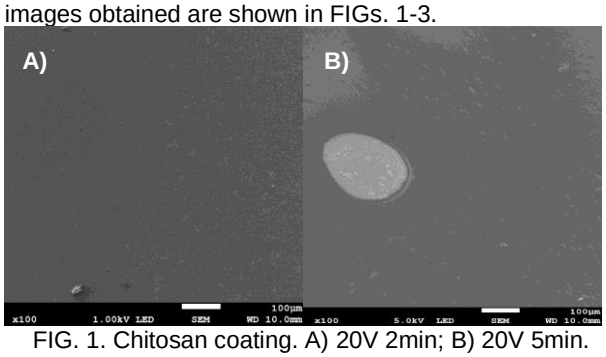

FIG. 1. Chitosan coating. A) 20V 2min; B) 20V 5min.

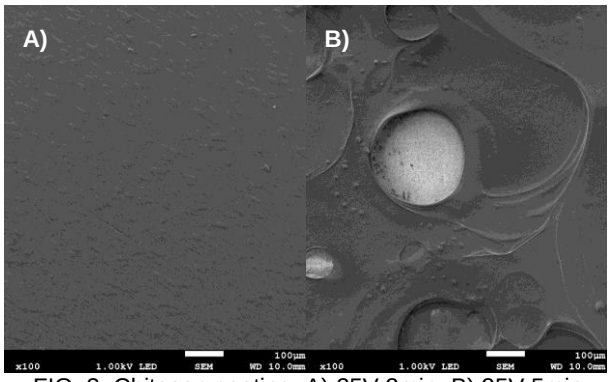

FIG. 2. Chitosan coating. A) 25V 2min; B) 25V 5min.

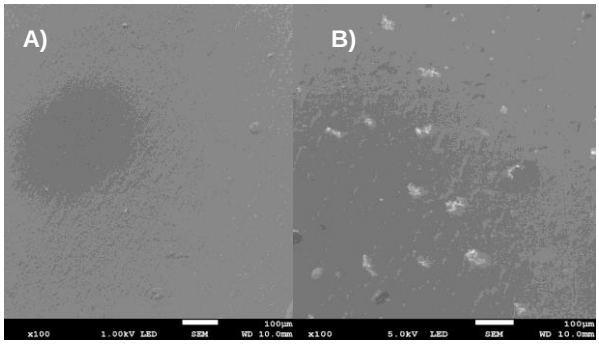

FIG. 3. Chitosan coating. A) 30V 2min; B) 30V 5min.

Nanoscratch test

Nanoscratch tests were conducted with a nanoindenter (NanoTest Vantage, Micro Materials) equipped with a Berkovich three-sided pyramidal diamond indenter. The scratch tests were repeated 10 times while increasing the load from 0mN to 400 mN at the load rate of 2.0 mN/s at a distance of 500 µm. The location of delamination was determined, based on SEM images and changes in frictional force during the test. The results are presented in TABLE 2. Uncertainties were estimated from standard deviation and experimenter uncertainty (15 µm).

TABLE 2. Nanocratch test results.

Parameters	Critical load (mN)
20V 2min	103.3 ± 20.3
20V 5min	315,8 ± 34.5
25V 2min	138.9 ± 24.9
25V 5min	Not performed because of bubble structure
30V 2min	247.0 ± 27.6
30V 5min	163.9 ± 20.9

Conclusions

Most of the coatings were characterized by a continuous, homogeneous structure. 20V 5min had single discontinuities. At 20V 5min, and 25V 2min bubble structure appeared, near the edge of the sample. Only chitosan deposited at 20V 5 min was characterized by a bubble structure. All samples except the coating deposited at 20V 2 min were hydrophilic. The best adhesion was characterized by the sample: 20V 5min, but the worst 20V 2min.

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IN VITRO BIOACTIVITY, CYTOBIOCOMPATIBILITY AND ANTIBACTERIAL PROPERTIES OF CHITOSAN/BIOGLASS COMPOSITES MODIFIED BY PEPTIDES

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Introduction

The basic requirements for biomaterials are biocompatibility, bioactivity and easy availability. Such properties have for example chitosan - a natural polymer that is biodegradable and inhibits the growth of microorganisms [1]. It is often used in the form of porous scaffolds, where it is often admixed with fillers, e.g. ceramics or bioglasses, to ensure better properties. Chitosan/bioglass composites can also be carriers for active compounds, such as peptides [2].
The aim of the work was to obtain and characterize chitosan composite materials with the participation of bioglass and peptides, in which the components would provide valuable biological properties.

Materials and Methods

The composites were obtained by thermally induced phase separation, from chitosan (HMC+) with a degree of deacetylation DD>92.6% and bioglass from the SiO_2-CaO-P_2O_5 system with the addition of ZnO or SrO. The composites were surface modified with selected peptides with pro-regenerative or antibacterial properties.
Microstructure and bioactivity of the composites were determined based on imaging and SEM/EDS analysis and comparison of the intensity of signals coming from Ca and P in relation to the intensity of the signal from Si of samples before and after incubation in SBF.
Cytotoxicity and proliferation were tested indirectly based on ISO 10993-5 guidelines on the human osteoblast cell line hFOB (ATCC) using the LDH (Roche) and WST-1 (Abcam) assay, respectively.
Antibacterial properties were determined according to the ASTM E2180-07 2012 standard using the Gram-positive bacteria *Staphylococcus aureus* PCM 2602 and the Gram-negative bacteria *Pseudomonas aeruginosa* PCM 2563, in relation to the porous structure obtained from pure chitosan.

Results and Discussion

Chitosan composites with a porous structure, with the pore size in a range of 40-140 µm, enriched with bioglass and peptides, were obtained in the work (FIG. 1).
The presented composites showed bioactivity in the *in vitro* study after 1-4 weeks of incubation in SBF (FIG. 2).
The obtained biocomposites did not show cytotoxic properties against human hFOB osteoblasts (ATCC) and showed proliferative properties at the level of about 150% in relation to control cells being not in contact with the extract from the composite. The presented composites showed antimicrobial activity against selected bacterial strains, achieving a reduction in the number of bacteria at the level of 75-98%.

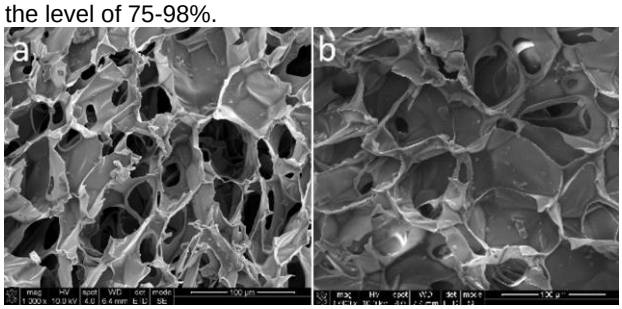

FIG. 1. Microstructure of composites: (a) chitosan/ bioglass with SrO, modified by proregenerative peptide; (b) chitosan/bioglass with ZnO, modified by antibacterial peptide.

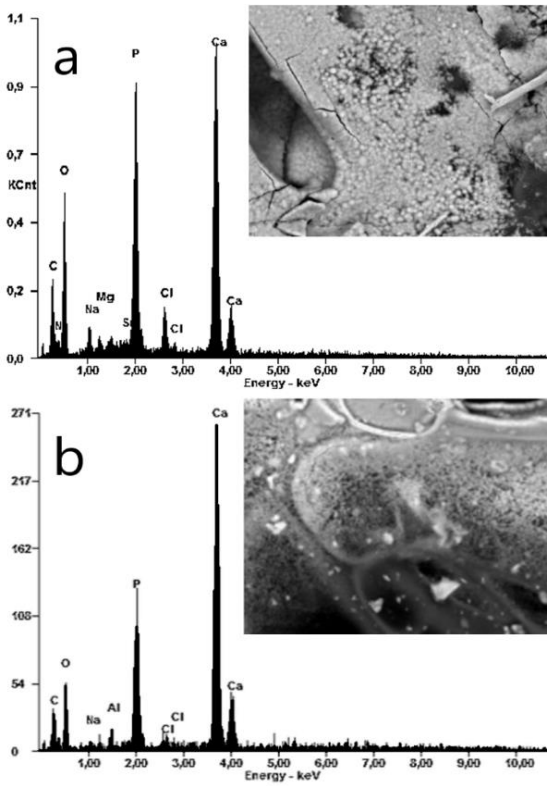

FIG. 2. In vitro bioactivity of composites: (a) chitosan/ bioglass with SrO, modified by proregenerative peptide; (b) chitosan/bioglass with ZnO, modified by antibacterial peptide.

Conclusions

The obtained porous chitosan composites are bioactive, and cytobiocompatible with human osteoblast. They have also antibacterial properties against various bacterial strains. The results allow to conclude that the obtained composites may be an attractive material for potential applications in regenerative medicine of bone tissue.

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IN VITRO BIOLOGICAL ACTIVITY OF SrO-DOPED BIOGLASS FOR MULTIFUNCTIONAL CHITOSAN COMPOSITES

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Introduction

The chemical composition of the glasses affects the release of ions that play an important role in the healing and regeneration of bone after implantation. Strontium has the ability to activate osteoblasts and inhibit osteoclast activity [1]. Sr^{2+} ions released from bioglass have osteoinductive effect [2] and antibacterial properties [3]. The aim of the study was to obtain bioactive, osteoinductive and antibacterial glasses using the sol-gel method for multifunctional composites.

Materials and Methods

The chemical formulations of bioglass were developed in the CaO-SiO_2-P_2O_5 system. Bioglass of 70 wt% SiO_2, 5 wt% P_2O_5 and 25 wt% CaO was used as a basic material (P5). In the basic formula of bioglass, 2 wt% (P5Sr2) or 5 wt% (P5Sr5) of CaO was replaced with SrO. Having performed the reaction mixtures from sol to gel and after the drying process was completed heat treatment at 650°C for 15 h was performed. The samples were prepared in two grain sizes I and II.

The biological tests were carried out in accordance with PN-EN ISO 10993, on the hFOB 1.19 human osteoblast cell line. Cell proliferation and cytotoxicity was determined by LDH and WST-1 tests. To determine antimicrobial properties of bioglasses cell cultures *Staphylococcus aureus* PCM 2602 and *Pseudomonas aeruginosa* PCM 2563 were used. Collected data were analysed and visualized using GraphPad Prism 8 (GraphPad Software, USA). Due to the limited number of experimental samples, normality of results distribution could not be confirmed. Thus, statistical calculations for different amounts of data obtained in the experiments were performed with the use of Mixed-effects Model which is based on Restricted Maximum Likelihood (REML) calculations (p = 0,05). In the next step, to control the false discovery rate, Benjamini, Krieger and Yekutieli multiple comparison test (p = 0,05) was carried out.

Results and Discussion

The results of in vitro biological tests are presented in FIG.s 1-3.

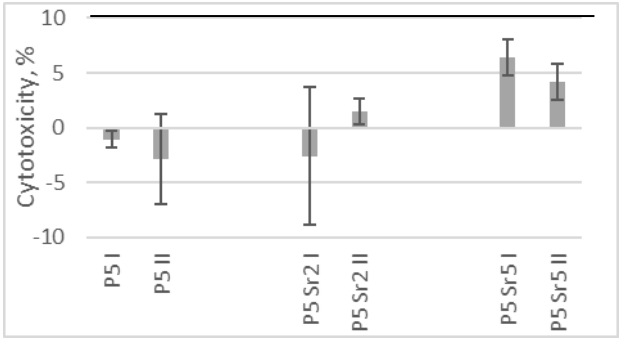

FIG. 1. Bioglass cytotoxicity after 48 h.

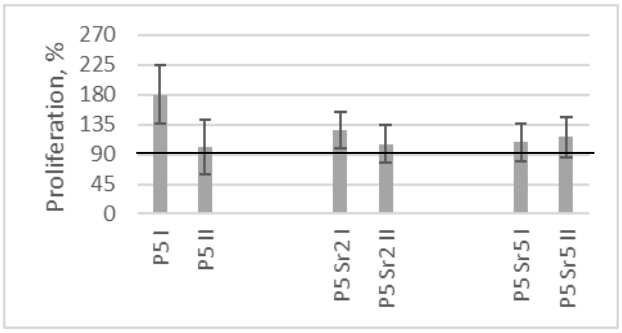

FIG. 2. Proliferation of the hFOB cell line after 48 h of contact with bioglass.

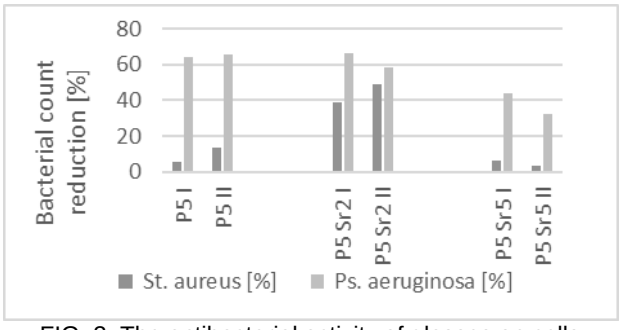

FIG. 3. The antibacterial activity of glasses on cells *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The proliferation of all tested glasses was higher than the required level of 90%. The highest antibacterial effect in contact with *St. aureus* showed bioglass containing 2% SrO. The level of their antibacterial activity depended on the type of bacterial strain.

Conclusions

The rate of bactericidal reduction of the obtained glasses depended on their chemical composition and the susceptibility of the cell culture.

Acknowledgments

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WHAT ABOUT THESE WOUNDS - WHAT DRESSING TO CHOOSE AND HOW THEY DIFFER?

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Introduction

Dressing materials belong to the group of medical devices (MD). This means that in order to be able to introduce such a device to the market, the manufacturer or its authorized representative, independently or with the participation of a notified body, must classify such a device in accordance with the requirements of the Medical Devices Act [1], the Regulation of the Minister of Health [2], and also guarantee that such a device meets a number of normative and legal requirements contained in the new requirements of the Medical Device Regulation (MDR) [3]. Despite legal regulations and normative requirements for dressing materials, as well as in accordance with national guidelines and recommendations [4-6], European [7,8] and British - NICE [9], the differences in the properties of individual materials are huge, even among dressings from the same group. The new regulations introduce the obligation to edit leaflets added to medical devices (IFU) in a clear and accessible way, understandable to a person who does not have specialist medical knowledge.
In the work carried out as part of the project „*O rany, co na te rany, czyli jaki opatrunek wybrać i czym właściwie się one różnią?*", commercial products of wound dressings from all key groups and types of dressing materials (intended for hard-to-heal wounds) selected in consultation with experts form Polish Wound Management Association (PTLR), were analysed.

Materials and Methods

Free swell absorptive capacity was tested according to PN-EN 13726-1:2005.
Antibacterial activity assessment was carried out in line with AATCC Test Method 147-2011 Antibacterial Activity Assessment of Textile Materials:Parallel Streak Method, against *Escherichia coli* ATCC 11 229, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10259.
Quantitative chemical analysis was performed on the basis of series of standards PN-EN ISO 1833 and Regulation (EU) No 1007/2011, Annex VIII.
Microscope images were taken using Motic SMZ-143-N2GG stereo microscope equipped with Motic Moticam 2500 camera.

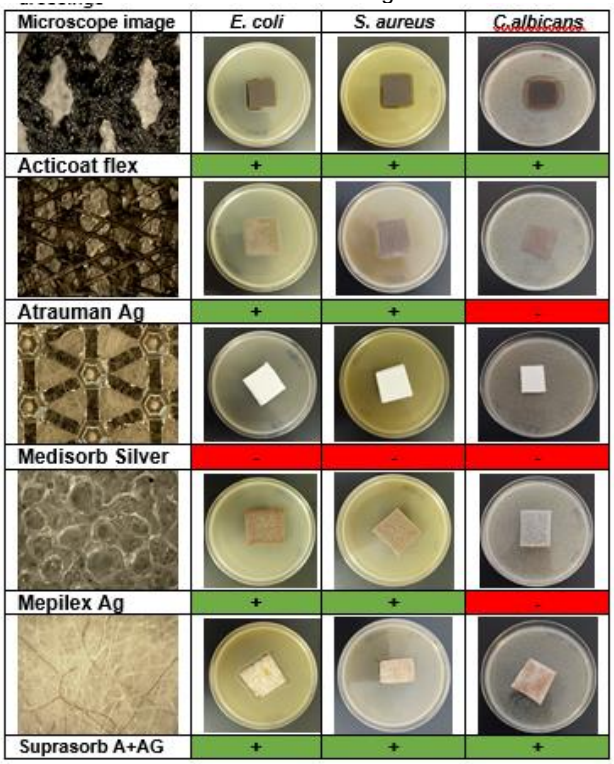
Results and Discussion

Determination of the antibacterial activity of samples of dressings containing diffuse active substance revealed that not all of the dressings exhibit antibacterial properties, in performed test method (TABLE 2). All samples contain silver as an active agent.

TABLE 1. Free swell absorptive capacity of wound dressings.

Free swell ab.capacity, g/100cm^2	Acticoat flex	Atrauman Ag	Medisorb Silver	Mepilex Ag	Suprasorb A+AG
	14,368 ± 0,474	4,464 ± 0,08	129,496 ± 1,273	280,564 ± 4,276	81,596 ± 3,544

TABLE 2. Antibacterial activity assessment of wound dressings.



Conclusions

Antibacterial activity of wound dressing can be assessed using multiple of different method. Failure of some of the samples in AATCC Test Method 147-2011 testifies to that active agent do not diffuse to the external environment. In order to check antibacterial activity of commercial products further antibacterial test will be carried out. As for the free swell absorptive capacity it corresponds strongly with the structure and raw materials composition of the tested dressings.

Acknowledgments

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RELEASE OF DRUGS USED IN THE TREATMENT OF OSTEOPOROSIS FROM IMPLANTS CONTAINING ZEOLITES WITH DIVALENT CATIONS

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Introduction

Osteoporosis is a disease that reduces bone mineral density, so bones are more fragile. The most popular drugs to prevent osteoporosis are bisphosphonates (BPs). Several methods of BPs delivering have been developed, but each has drawbacks. When BPs are orally administered absorption of the drug is very low, and side effects are heartburn, osteonecrosis of the jaw and nausea [1]. After intravenous administration, flu-like symptoms occur. The side effect of the intranasal and transdermal application is local toxicity. Therefore, a new way of delivering these drugs is sought to improve the living conditions of people who have osteoporosis. An interesting alternative is a drug-containing material that will be introduced as an implant near lesions. It cannot be every material because its most important feature is that it should release the drug slowly. Materials with divalent cations have a high potential as carriers of BPs. This is because there are strong interactions between divalent cations and the BPs [2]. Until now, drug carriers that consisted of calcium phosphates (e.g. hydroxyapatite) have been used. However, materials of this type have disadvantages. The amount of drug released from its surface is very low. Because they contain so many calcium, and their structure is similar to bones, they resorb the drug, and thus the effectiveness of the treatment is lower. Due to this, zeolites seems to be an ideal alternative. Divalent cations present in the zeolites can be removed by ion exchange with potassium and sodium ions from body fluids, thanks to which the interaction between the drug and the carrier disappears. Due to the disappearance of interactions on these materials, the resorption process would not take place. Furthermore, since the ions supplied from the body fluids would affect the exchange gradually, the drug would also be released slowly. Moreover, due to the binding of phosphonium groups that are responsible for the toxicity of BPs, local inflammation should not occur. The slow release confirm the great potential of these materials in the controlled release of drugs for osteoporosis.

Materials and Methods

The methodology for the preparation of a titanium implant modified with a zeolite layer was described in publication [3]. The methodology for the preparation of a chitosan scaffold containing zeolite was described in [4]. Both materials contained zeolites in calcium form. Both materials were placed in the risedronate solution for drug sorption. The next step was drug release under the influence of simulated body fluids. The amount of released drug was determined by UV-Vis spectroscopy. After measuring the amount of drug released each day, the simulated body fluid was replaced with a new one to provide new ions.

Results and Discussion

As can be seen in FIG. 1, both drugs are released in controlled low doses. Drug release from the chitosan-zeolite scaffold was carried out for 30 days. Release from this material continued, but the doses were so small that it was not possible to determine them using the apparatus used. The release was studied longer for the titanium alloy modified with calcium zeolite. The release for this material lasted about 220 days and it should be noted that the drug is released further.

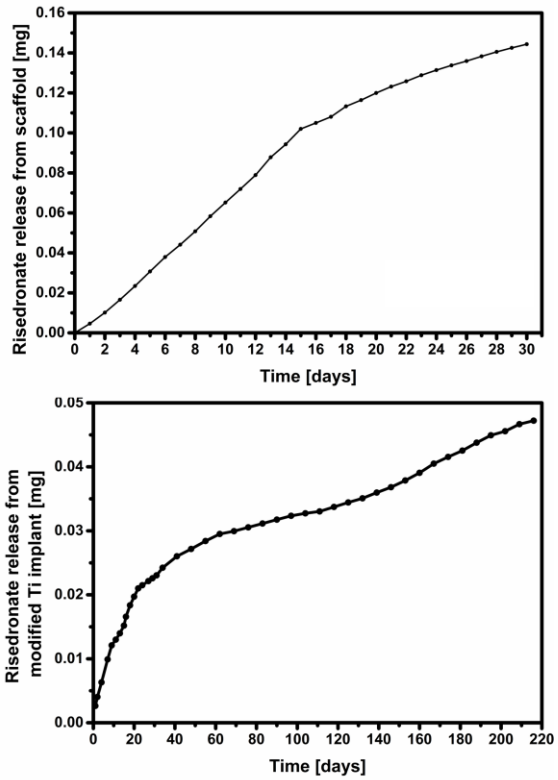

FIG. 1. Release of an osteoporosis drug (risedronate) from a chitosan-zeolite scaffold and a titanium alloy modified with a zeolite layer.

At the moment, the literature has not described a drug delivery system for osteoporosis in the form of implants that release the drug for so long. Importantly, the drug is released in controlled manner and there is no "burst release" effect.

Conclusions

Studies prove that zeolites containing calcium ions can be used as carriers of drugs for osteoporosis. In addition, the release of the drug from this type of materials is slowed down, which indicates their high application potential. Implants of this type can be used when a bone fragment needs to be removed and can contribute to faster recovery by the patient.

Acknowledgments

This work was produced with financial support from the Polish National Science Centre (grant number UMO-2020/39/B/ST5/00320).

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TITANIUM IMPLANT MODIFICATION WITH MOF LAYER – CHARACTERIZATION OF THE MATERIAL BY FT-IR MICROSCOPY

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Introduction

Bone diseases affect a large proportion of the world's population. They often lead to the occurrence of serious bone fractures, which make it necessary to replace them with artificial implants [1]. The main material used to create implants is titanium, more precisely its alloy Ti6Al4V. This alloy has excellent functional properties such as: biocompatibility and corrosion resistance. It also poses excellent osteointegration capacity which is key to long term implant stability [2]. Implants made of this material also have a survival rate of more than 90% in the first 5 years after implantation. However, the use of materials made of titanium in medicine also has some disadvantages [3]. Titanium is a bioinert material, so it does not cause allergies, but the human body treats it as a foreign body. Therefore, osseointegration is slowed down and the surrounding tissues may become inflamed [1]. The next major problem is the occurrence of bacterial or fungal infections, which are the greatest threat to the elderly undergoing implant surgery [4]. The solution to this problem is the functionalization of the implant surface. One of the promising materials for this purpose are Metal Organic Frameworks (MOFs). They have many biomedical applications such as: drug delivery systems or titanium implant modification. However, so far, MOFs with synthetic linkers have been used to modify implants [5]. Gallic acid is a natural phenolic acid commonly found in plants. It has been proven to have anti-oxidative, anti-inflammatory and anti-cancer properties. There are also reports on its antibacterial activity [6]. It has found application in the synthesis of MOFs. In this work, we synthesized Zinc gallate MOF on the surface of a titanium implant to create an antibacterial layer which enhanced bioactivity. Both zinc and gallic acid have an antibacterial effect, which allowed to obtain an active layer against microbes.

Materials and Methods

The methodology for the preparation of a titanium implant modified with gallic acid based MOF layer was similar to that described in publication [7]. The material was characterized using various research techniques including SEM/EDS. Water contact angle of the materials at various stage of the synthesis were measured. In-vitro bioactivity and antimicrobial properties of the prepared layer was also assessed. The most important technique used was FT-IR microscopy. It was used to test the uniformity of the implant coverage and to examine the results of the bioactivity study.

Results and Discussion

The effective synthesis of the obtained layer was confirmed by various research techniques, including FT-IR spectroscopy, FIG. 1. As you can see the spectrum after the final stage has new bands.

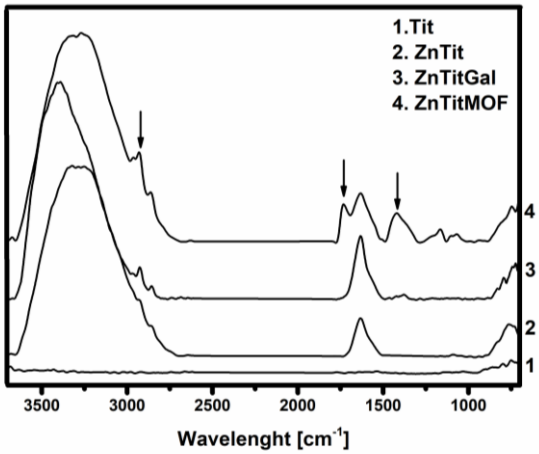

FIG. 1. FT-IR spectra of the prepared material at various stage of the synthesis.

The antimicrobial tests performed show that titanium modified with a MOF layer based on gallic acid has antibacterial and antifungal properties. Their results are presented in TABLE 1. The highest growth inhibition ability was observed against gram-positive bacteria and the lowest against gram-negative bacteria, which may be related to differences in cell structure.

TABLE 1. Antimicrobial activity expressed as the optical density of the suspension of microorganisms (OD600) after 24 hours of cultivation in the presence of the tested materials.

Studied microorganisms	Optical density (OD600) in the presence of the tested materials		
	Tit	ZnTit	ZnTitMOF
Pseudomonas aeruginosa	1.12 ± 0.17	1.04 ± 0.18	0.89 ± 0.07
Escherichia coli	1.16 ± 0.19	0.96 ± 0.13	0.77 ± 0.16
Staphylococcus aureus	1.15 ± 0.11	1.03 ± 0.17	0.67 ± 0.08
Bacillus cereus	0.95 ± 0.07	0.93 ± 0.13	0.71 ± 0.03
Candida albicans	1.07 ± 0.11	0.94 ± 0.11	0.74 ± 0.08

Conclusions

Studies prove the effectiveness of the synthesis of gallate based MOF layer on titanium implant. It was proved that the obtained layer posses antibacterial and antifungal properties. Conducted studies show also excellent bioactivity of the prepared layer. To the authors best knowledge this is the first modification of titanium implant with MOF layer based on natural linker. Obtained coating can be used to prevent biofilm formation and eliminate the risk of infection of titanium implants.

Acknowledgments

This work was produced with financial support from the Polish National Science Centre (grant number UMO-2020/39/B/ST5/00320).

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BIOINSPIRED AND BIOLOGICALLY ACTIVE HYDROGELS AND THEIR POTENTIAL USE FOR BIOMEDICAL APPLICATIONS

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Introduction

Nature is a great source of both inspiration and material components. Additionally, we have a variety of synthetic ones to choose from. Both groups have their pros and cons and often the best properties are achieved only when bio-derived and man-made materials are combined. The situation is not different when it comes to hydrogels. Here, one of the most crucial advantages is the high amount of water, making them similar to many biological systems. Biologically active compounds introduced into the base system will additionally boost, already pretty good, biological properties, while addition of synthetic polymer network should be beneficial in terms of e.g. mechanical properties.

In the current study, particular attention was paid to poly(vinyl alcohol) (PVA), chitosan (CS), and hyaluronic acid (HA). HA is a natural component of the extracellular matrix (ECM). CS, derived from chitin, offers many interesting characteristics like antimicrobial activity or wound healing promotion. PVA is a water-soluble synthetic polymer with tunable mechanical properties, ease of modification, and versatility.

These three polymers were tested in different combinations for two possible applications, i.e. tissue engineering of osteochondral defects and wound healing. For the first one, kartogenin (KGN) – a small molecule known for its ability to induce chondrogenic differentiation of mesenchymal stem cells, and hydroxyapatite (HAp) – ceramic material similar to mineral bone component, were used. For the latter, the selection criteria were based on anti-inflammatory, soothing, and cell proliferation-promoting properties, hence pomegranate peel extract (PGP), tea tree oil (TTO), and allantoin (All) were chosen. The hydrogels were crosslinked without any chemical agents, using the freeze/thaw method. The materials were tested in terms of their structure, microstructure, chemical stability, wettability, water absorption ability, bioactive compounds release, and cytotoxicity.

The results confirmed that PVA/CS and PVA/HA systems constitute promising hydrogels matrices for further modification. Thanks to entirely safe crosslinking method (freeze-thaw), no toxic reagents were used, while chemical stability was achieved, as shown in incubation assays. As expected, mechanical properties of the double-network systems were improved. All of the modifiers: KGN, HAp, PGP, TTO, All affected final characteristics of the hydrogels, with particular influence on biological behaviour.

Among many different compositions tested within the study, the most beneficial for future application in ostechondral defects regeneration and wound healing were selected. Those will be further evaluated and optimized.

Acknowledgements

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OVERCOMING PHOTOINITIATOR LIMITATIONS. SELF-CROSSLINKING MATERIAL FOR BIOPRINTING APPLICATION

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Introduction

Main aim of presented research is to develop photocurable material basing on gelatin derivatives which do not demand polymerization initiator for efficient crosslinking of polymer fibers. One of the stages of creating 3D scaffolds from hydrogels is polymer cross-linking using a photoinitiator such as LAP or Irgacure. It is commonly known that the by-products of photoinitiator degradation show cytotoxicity, which is a very undesirable effect in biomaterials engineering.[1]

Facing the problem of photoinitiators cytotoxicity we initiated research on materials capable of crosslinking without using of photoinitiator (FIG. 1). The key of the research was to find compounds containing appropriate groups sensitive to UV-Vis irradiation, incorporated them into polymer structure and optimize working parameters for application in bioprinting.

In order to create new materials, a series of reactions was carried out to create active esters of appropriate acids and attach them to gelatin. Due to presence of functional groups such as primary amine groups and hydroxyl groups in the gelatin structure, it was possible to functionalize peptide chains.[2,3] The reaction procedure involved the preparation of active esters of carboxylic acids using commonly known coupling reagents such as EDC or DCC which are widely used in peptide chemistry.

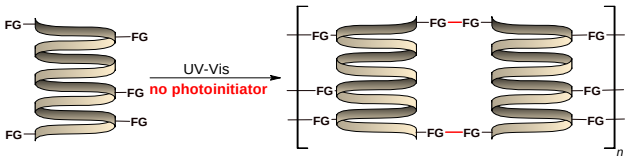

FIG. 1. Polymer cross-linking.

Results and Discussion

As a result of the conducted experiments a wide group of materials was obtained. These materials can crosslink under the UV-Vis irradiation using a selected wavelength. In addition, they do not require the use of any photoinitiator, and the cross-linking process is caused by the reaction within the substituents. Optimization of the synthesis reaction allowed to regulate the degree of substitution in the range of 20-100%

Conclusions

The use of gelatin fibers as the basic biopolymer paved the way for the new group of materials expansion. It is possible to find new applications for the above materials, which would allow for a significant development of the biomaterials chemistry. In addition, using various synthesis methods, it is possible to functionalize the most important part of the polymers in bioprinting such as chitosan or hyaluronic acid.

Acknowledgments

TECHMATSTRATEG-III/0027/2019-00/National Centre for Research and Development

Conflicts of Interest

Michal Wszoła is the co-founder of Polbionica Ltd.

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RESVERATROL - THERAPEUTIC POTENTIAL

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In recent years, research have been directed toward the development of new drug forms that provide improved stability and increased pharmaceutical and bioavailability. Due to their wide range of biological activities, flavonoids are a very interesting group of natural compounds that are used as active substances encapsulated in a hydrogel matrix. Their chemical structure and the presence of various groups and groupings in their molecules make them exhibit high biological activity and determine various effects on cellular metabolism [1]. Resveratrol is one of the flavonoids, found mainly in fruits and their preparations, especially in grapes and in high concentrations in red wine. This compound has a wide range of effects on the human body both in the prevention and treatment of various diseases. it has a beneficial effect on the circulatory system. In vitro studies have shown that it inhibits the development of cancerous processes. Moreover, the compound protects against neurodegenerative diseases, has antioxidant, antiproliferative, anti-inflammatory and anti-angiogenic effects [2].
Due to the established effect of resveratrol on the process of wound healing, the direction of research is the development of technology for the preparation of modern dressing materials. In addition to its anti-inflammatory properties, the potential usefulness of resveratrol in wound healing is evidenced by its effect on fibroblast proliferation and its ability to regulate the expression of vascular endothelial growth factor protein, responsible for the initiation of granulation tissue formation and angiogenesis. the introduction of trans-resveratrol into a hydrogel dressing creates the possibility of controlled release of the active substance at the site of application [1].

Acknowledgments
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 "Polymeric Biomaterials with a character of active substance carrier for medical applications, nr SKN/SP/568478/2023.

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MANGANESE AS A MODIFIER OF CALCIUM PHOSPHATE BIOCERAMICS

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Introduction

Manganese (Mn) is a chemical element that can be used in the modification of calcium phosphates. Manganese has the ability to oxidize calcium phosphates by providing additional manganese ions in higher oxidation levels. This process can alter the structural and physicochemical properties of calcium phosphates. In addition, manganese can act as a catalyst in calcium phosphate modification reactions, accelerating chemical processes and affecting the structure of the final product. For example, manganese can activate enzymes that participate in the modification of calcium phosphates in organisms. Next, manganese is an essential micronutrient for living organisms, including humans. It plays an important role in many biological processes, such as calcium metabolism, collagen synthesis and nervous system function. The use of manganese in the modification of calcium phosphates may be related to its influence on the biological activity of these compounds. Manganese may also affect the structural stability of calcium phosphates, preventing the degradation or breakdown of these compounds. Thus, manganese-mediated modification can lead to durable, strong and more functional phosphate materials. Note that the specific effect of manganese on the modification of calcium phosphates may depend on a number of factors, such as reaction conditions, manganese concentration, presence of other chemicals, etc. Additional studies and experiments are usually needed to thoroughly understand and utilize the properties of manganese in the modification of calcium phosphates [1,2].

Materials and Methods

Modification of calcium phosphates with manganese can be accomplished using a variety of materials and methods. One way to modify calcium phosphates with manganese is to add manganese ions to the chemical reaction leading to the synthesis of calcium phosphates. Depending on the desired properties of the final product, the concentration of manganese in the reaction, pH, temperature and other process parameters can be adjusted. Manganese can act as a catalyst in calcium phosphate modification reactions. It can be used to activate enzymes involved in these processes. The manganese catalyst can accelerate the reaction, change the rate and yield of chemical transformations, and affect the structure and physicochemical properties of calcium phosphates. Manganese nanoparticles, on the other hand, can be used as a modifier of calcium phosphates. These nanomaterials can be introduced into the phosphate matrix to influence its structure, mechanical, thermal or optical properties. Manganese nanoparticles can be synthesized by various methods, such as chemical, physical or biological methods. Also known is the impregnation method, which involves impregnating a phosphate matrix with a manganese-containing compound, such as manganese salt. Through the impregnation process, manganese can be introduced into the structure of calcium phosphate, which can lead to changes in its physical and chemical properties [3].

Results and Discussion

Modification of calcium phosphates with manganese can be accomplished using a variety of materials and methods. One way to modify calcium phosphates with manganese is to add manganese ions to the chemical reaction leading to the synthesis of calcium phosphates. Depending on the desired properties of the final product, the concentration of manganese in the reaction, pH, temperature and other process parameters can be adjusted. Manganese can act as a catalyst in calcium phosphate modification reactions. It can be used to activate enzymes involved in these processes. The manganese catalyst can accelerate the reaction, change the rate and yield of chemical transformations, and affect the structure and physicochemical properties of calcium phosphates. Manganese nanoparticles, on the other hand, can be used as a modifier of calcium phosphates. These nanomaterials can be introduced into the phosphate matrix to influence its structure, mechanical, thermal or optical properties. Manganese nanoparticles can be synthesized by various methods, such as chemical, physical or biological methods. Also known is the impregnation method, which involves impregnating a phosphate matrix with a manganese-containing compound, such as manganese salt. Through the impregnation process, manganese can be introduced into the structure of calcium phosphate, which can lead to changes in its physical and chemical properties [4].

Conclusions

The use of manganese in the modification of calcium phosphates can lead to significant changes in their physicochemical, mechanical, biological and thermal properties. The addition of manganese can increase mechanical strength, improve biocompatibility, control ion release, affect thermal stability and modify the optical properties of calcium phosphates. These research results indicate the potential for manganese application in various fields such as biomedicine, materials engineering and optics. Nevertheless, further research, experimentation and analysis are needed to thoroughly understand manganese's mechanisms of action and optimize the modification of calcium phosphates.

Acknowledgments

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SILYMARIN - THERAPEUTIC POTENTIAL

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Introduction

Flavonoids are a diverse collection of compounds belonging to groups with a polyphenolic structure. Extracted from plants, fruits and vegetables, they are characterized by antioxidant, anti-inflammatory and anticancer properties. As a result, they have found applications in medicine and the pharmaceutical industry [1]. A popular compound is flavonolignan, which includes silymarin. Extracted from the husks of the seeds of the spotted thistle, its composition includes slibin, isosylibin, silydianin and silicristin. Slibin reacts with oxygen free radicals and reduces glutathione oxidation in the liver and mitochondria. Silymarin has a regenerative effect on liver cells and reduces the effects of tumor agents. Its properties also include poor water solubility, resulting in poor absorption [2,3]. However, administration of flavonolignan in hydrogel form increases bioavailability.

A pH-responsive hydrogel material based on alginate, poly(d,l-lactic-co-glycolic acid) (PLGA) and silymarin was developed. The material was tested in vitro. The aim of the study was to test the effect of the hydrogel material after oral administration on changing the bioavailability of silymarin. Degradation studies confirmed the positive effect of the hydrogel. Silymarin was released for a long time. Such an effect allows to increase the bioavailability of the flavonoid [4]. The combination of polymers with silymarin also helps in wound healing in diabetic patients. Xanthan gum was combined with silymarin to form hydrogel wafers. These were subjected to a freeze-drying process. Cell migration studies showed that the produced wafer had an inhibitory effect on endothelial cell migration induced by high concentrations of glucose [5].

Conclusions

Based on press reports, the combination of silymarin with polymers has been shown to increase its bioavailability, resulting in a better effect after oral administration. In addition, studies show a positive effect of such a hydrogel material on wound healing in diabetic patients.

Acknowledgments

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QUERCETIN – ITS THERAPEUTICAL POTENTIAL

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Introduction

Quercetin is a natural flavonoid widely present in plants we use every day f.e. onions, grapes or even tomatoes. Its medical potential has been observed because of its presence in medical plants. If a substance can be found in medical plant, it means it can also be used in medicine as a single substance. The presence of a phenolic hydroxyl group in quercetin and double bonds endows it with a strong antioxidant activity. This activity can be used in treatment of cardiovascular diseases and cancer. But most importantly in addition to its antioxidant activity, it has also shown a significant antibacterial properties [1]. Both characteristics of quercetin makes it a promising active substance in skin treatments like skin cancer. It can be delivered through hydrogels. Hydrogels are polymeric biomaterials that swell when exposed to water and form a three-dimensional structure with multiple internal pores [2]. In these pores there can be carried an active substance which is released when hydrogel swells. Quercetin carried by hydrogel can be applied directly to the site of disease while hydrogel protects the wound from the external conditions.

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The research work was carried out within the SMRT_MAT Functional Materials Science Club, BioMat section at the Faculty of the Cracov University of Technology within the framework of “Polymeric Biomaterials with a character of active substance carrier for medical applications”. SKN/SP/568478/2023

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HAp POWDERS AS A CARRIER OF BIOLOGICALLY ACTIVE SUBSTANCES

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Introduction

Synthetic CaPs are frequently used as materials for filling bone defects, while their porous structure allows modification with active substances, and thus subsequent use as a drug carrier for controlled release. An advantage of CaPs in the context of their use as biomaterials, including drug carriers, is their biocompatibility, as they do not induce inflammation or other negative reactions. The methods of their synthesis makes it possible to adjust the size and structure of the grains for drug loading[1]. The diffusion of active substances through the ceramic pores depends on their concentration gradient, solubility, the porosity of the ceramic carrier itself as well as the specific surface area and size[2,3].There are several types of CaP, which differ in the molar ratio of calcium and phosphorus (Ca/P). A lower Ca/P ratio corresponds to more acidic and relatively water-soluble phases[4]. Currently, hydroxyapatite (HAp) and tricalcium phospahte (TCP) are widely used as implant materials[5,6]. HAp is the most well-known and widespread phase and has a Ca/P ratio of 1.67. It represents the mineral part of natural bone, which consists of 70% of just this inorganic material[7]. TCP has a Ca/P ratio of 1.5 and occurs in two polymorphic varieties[8]. Furthermore, also of interest in the context of applications where the implant is to be replaced by newly formed bone over time is brushite (DCPD), which is 100 times more soluble in body fluids than HAp. For this reason, brushite are resorbable in vivo faster, and this aspect, also affects the increase in porosity of such a biomaterial over time, which allows surrounding tissues to grow faster into it[9,10]. In this study, four different ceramic powders were compared, commercial hydroxyapatite (c-HAp) and TCP, brushite as well as s-HAp obtained by wet precipitation method. Next, they were modified with clindamycin using physical sorption method. Using high-performance liquid chromatography (HPLC), the drug release rate was determined. The clean powders were subjected to physicochemical analysis including X-Ray diffraction analysis (XRD), Brunauer, Emmett, Teller (BET) Specific Surface Area (SSA) and Porosity, Fourier Transform Infrared Spectroscopy (FTIR) and Ca/P molar ratio.

Materials and Methods

Three of the ceramic powders were obtained by wet precipitation methods with different Ca/P molar ratios in the range of 1.0-1.67, and a commercial hydroxyapatite was selected as a reference powder. The molar ratio of Ca and P (Ca/P) was determinate in accordance with the Polish standard, for phosphorus based on PN-80/C-87015 for calcium based on PN-97/R-64803[11,12].The absorbance at 430 nm was measured using a UV-vis spectrophotometer. Structural characterization performed using a XRD. Individual functional groups were identified using FT-IR. The SSA of the samples was determined with the multipoint BET analysis method, using Auto-sorb-1 Quantachrome flow apparatus, with nitrogen as an adsorbate, at-196 °C. The antibiotic selected for powder modification by physical sorption was clindamycin hydrochloride, Ceramic weights of 0.5 grams each were inserted into 40 mL of the drug solution at a concentration of 2 mg/mL stored at tightly closed containers at 4°C for 5 days. To examine the amount of clindamycin released from the samples, the collected incubation fluids were analysed by HPLC.

Results and Discussion

The determined Ca/P molar ratios differ slightly due to the incorporation of foreign cations or anions into the atomic structure of the ceramics. In the case of s-HAp and c-HAp, results are close to the stoichiometric HAp.

TABLE 1. Calcium and phosphorus content and Ca/P molar ratio in obtained powders (mean ± SD).

Sample	Ca content (wt.%)	P content (wt.%)	Ca/P molar ratio
s-HAp	37.51 ± 0.65	17.26 ± 0.19	1.67
c-HAp	37.46 ± 0.38	17.27 ± 0.12	1.67
TCP	36.93 ± 0.32	18.87 ± 0.23	1.51
Brushite	37.81 ± 0.41	27.91 ± 0.34	1.04

In SEM-EDX analysis a high similarity was observed between s-HAp and brushite (flocculent shape in small agglomerates). The most regular and granular shape was observed for TCP.

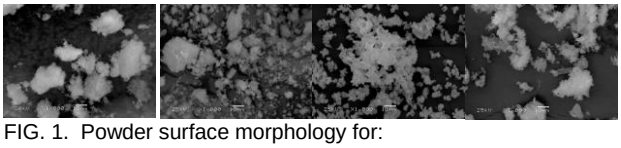

FIG. 1. Powder surface morphology for:
a) s-HAp b) c-HAp c) TCP d) Brushite

Importantly, the morphological properties such as high SSA, large pore volume, and narrow particle size distribution are well suited for the application of nanoencapsulation in drug delivery systems. The results obtained are presented in TABLE 2.

TABLE 2. N2 physisorption-derived parameters.

Sample	SSA [m2/g]	Pore Size Distribution DFT [nm]	Pore volume macropores [cm^3/g]	Pore Area micropores Vt [m^2/g]
s-HAp	53	4,10,11,13	0,345	3,52
c-HAp	64	11, 13, 17, 20, 22, 24, 28	0,27	0
TCP	61	8, 10, 11, 13	0,304	0
Brushite	32	4, 7, 8, 10	0,21	4,94

The amount of clindamycin in mg/mL released from the powder samples for c-HAp was too small to be determined. After a 14-day immersion, the largest amount of drug was released from brushite, and the amount is practically double that for s-HAp.

Conclusions

Selected methods of synthesis make it possible to obtain ceramic powders of TCP, brushite, and hydroxyapatite. This fact was confirmed by FTIR analysis, where the main functional groups present in the materials were assigned, as well as by XRD analysis, in which the obtained diffractograms were related to the corresponding data sheets. All powders were phase-pure; however, the observed background spectrum suggests that they are only partially crystalline and partially amorphous. A relationship was observed between the specific surface area of the ceramic and drug release. The smaller the specific surface area, the faster the drug is released.

Acknowledgments

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POLYMERIC SYSTEMS FOR CONTROLLED RELEASE OF ACTIVE SUBSTANCES

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Introduction

Albumin, a protein present in blood plasma, has many uses in medicine, including as a drug carrier. Its ability to bind different substances makes it an attractive carrier for drugs with diverse physicochemical properties [1]. Due to its properties, albumin has been used as a carrier for various drugs, including cytostatics. [2] Cytostatics are medicines used to treat cancers that inhibit the growth of and the division of cancer cells. The use of albumin as a carrier of cytostatic drugs aims to improve the availability and bioavailability of drugs in the body and minimize their toxicity to healthy tissues. [3] In addition, the use of albumin as an innovative carrier of cytostatic drugs reduces the occurrence of side effects often associated with chemotherapeutic treatment [4].

Materials and Methods

In the present work, albumin carriers were prepared by salt-induced precipitation technique and characterized by dynamic light scattering (DLS), UV-Vis spectroscopy and infrared spectroscopy with Fourier transform (FT-IR). Chitosan/gelatin based hydrogels containing protein carriers were then prepared by UV radiation. Further studies were aimed at analyzing physicochemical properties of prepared hydrogel materials, including determination of their ability to swell, characterization of their chemical structure using FT-IR technique and verification of their tendency to degrade in simulated physiological fluids. In addition, the mechanical properties of hydrogels were investigated and their surface morphology was assessed using scanning electron microscopy (SEM). In addition, the wettability of hydrogels and their ability to permanently release albumin have also been determined.

Results and Discussion

FIG. 1 shows the scheme of preparation of protein nanospheres. Albumin was obtained by salinating proteins.

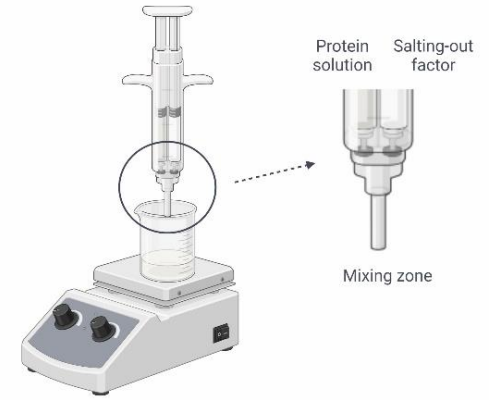

FIG. 1 Scheme for the preparation of protein nanospheres.

On the basis of the analysis, the sorption capacity of hydrogel materials was determined. All the samples received showed sorption properties which are important for the future use of these hydrogels as dressing materials. In addition, incubation studies in simulated body fluids showed no abrupt changes in pH values that could indicate degradation of polymeric materials. Spectroscopic analysis confirmed the presence of absorption bands characteristic of amino acids present in the protein, which was used as a modifier. In addition, the release profile of the active substance was determined and it was confirmed that under appropriate conditions the developed material allows simultaneous release of the therapeutic while maintaining its sorption properties.

Conclusions

The main application objective of the developed materials is their potential use as dressing materials supporting the treatment of tumors from the outside, i. e. as materials applied to the skin in the case of skin tumors. Albumin, as mentioned earlier, it is an excellent carrier of chemotherapeutic drugs, while hydrogel dressing supports the regeneration processes of wounds formed around the tumor tissue.

Acknowledgments

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EFFECT OF SYNTHESIS PARAMETERS ON PHYSICOCHEMICAL PROPERTIES OF CALCIUM PHOSPHATES

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Introduction

The challenge of modern medicine is undoubtedly the diseases of the 21st century, which are developing along with the progress of civilization. Among these diseases are cardiovascular diseases, cancer as well as osteoporosis. Osteoporosis is a systemic disease characterized by a decrease in bone mass and abnormalities in bone structure. It is initially asymptomatic, and often its first noticeable symptom is an osteoporotic fracture, resulting from an inappropriate event, such as a fall from one's own body. Very often osteoporotic fractures require surgical intervention, and due to the weakened bone structure, the implant materials used should be characterized by appropriate bioactivity, while supporting regenerative processes and reconstruction of the bone defect. In view of this, it is extremely important to research innovative materials that can be used in bone tissue regenerative medicine. The basic material that is often referred to as bone substitute material is hydroxyapatite as well as other varieties of calcium phosphates [1, 2]. Hydroxyapatite is one of the most important minerals in natural bone tissue. It can be used as a material for repairing and filling bone defects, such as fractures, bone defects, defects after tumor removal or defects resulting from bone disease. Hydroxyapatite acts as a scaffold that allows new bone tissue to grow, integrating with surrounding tissue and providing stable and durable bone reconstruction. Hydroxyapatite can also act as a drug carrier in bone regeneration therapy. Drugs such as growth factors, bone morphogenetic proteins (BMPs), antibiotics or other therapeutic agents can be incorporated into the hydroxyapatite structure. Then, after implantation, they are released gradually, providing locally necessary factors to stimulate bone regeneration, fight infection or accelerate the healing process [3, 4]. Hydroxyapatite is also used as a coating for orthopedic and dental implants, such as joint prostheses, screws, plates or dental implants. The hydroxyapatite coating improves adhesion and integration of the implant with bone tissue, leading to a faster healing process, reducing the risk of rejection and ensuring a permanent connection between the implant and bone. Hydroxyapatite can serve as a matrix for stem cells or progenitor cells. These cells can be implanted into the hydroxyapatite structure, allowing them to adhere, proliferate and differentiate into osteoblasts, the cells that form bone tissue. This is particularly important in the regeneration of larger bone defects, where more cells need to be delivered to rebuild bone tissue [5, 6].

Materials and Methods

In this study, the wet precipitation method, also known as the solution deposition method, is a commonly used technique for the production of calcium phosphates, including hydroxyapatite (HAp). The process involves a chemical reaction between solutions of phosphorus and calcium compounds in the presence of suitable reactants that lead to the formation of a calcium phosphate precipitate. During the synthesis, the influence of a number of different parameters was checked. The resulting HAp powders were analyzed using Fourier transform infrared spectroscopy, grain size was determined using dynamic light scattering technique and phase composition was determined by performing X-ray diffraction analysis.

Results and Discussion

A number of syntheses have been carried out to evaluate selected parameters on the physicochemical properties of calcium phosphate powders in particular hydroxyapatite. Relationships between synthesis parameters such as the concentration of reactants, the method of mixing, the pH of the reaction medium and the ratio of all substrates were determined. The type and ratio of reactants used had a particularly significant effect on the phase composition of the powders obtained. In turn, when different types of mixing systems were used, the product was obtained in different yields.

Conclusions

The choice of synthesis method, such as deposition method, sol-gel method, hydrothermal method, etc., can affect the structure, morphology and particle size of calcium phosphates. Different methods can lead to different physicochemical and structural properties of calcium phosphates. It is worth noting that the interactions between different synthesis parameters are complex, and the optimal synthesis conditions may vary depending on the intended use of calcium phosphates. Therefore, it is important to conduct studies and experiments to optimize the synthesis parameters and obtain the desired material properties.

Acknowledgments

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HYDROGEL TRANSDERMAL SYSTEMS IN ANTICANCER THERAPY

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Introduction

Hydrogels are three-dimensional networks of polymer chains, which, thanks to their porous structure, have the ability to absorb a large amount of water without dissolving in it. This is related to their construction, water retention is due to the presence of hydrophilic functional groups, while resistance to dissolution is due to cross-links between network chains. Hydrogel materials can be prepared from both natural and synthetic polymers. Synthetic polymers, however, have an advantage over natural ones due to long service life, high capacity of water absorption, and high gel strength. They also have well-defined structures that can be modified, so you can control relevant properties such as mechanical strength, biodegradability and response to chemical or physical stimuli. Hydrogels can be obtained in many ways, including: polymerization, parallel cross-linking of multifunctional monomers or by reacting polymers with suitable cross-linking agents [1]. Due to the high-water content, hydrogel materials have a degree of elasticity close to that of natural tissue. Due to the fact that hydrogels show good compatibility with drugs and are non-reactive and non-toxic, they can be safely used in pharmaceutical applications. Stimuli such as: pH, electrical signals, light or temperature can be used to trigger the release of drugs from the network [2]. The main advantage of transdermal drug delivery systems is the prevention of drug delivery to non-targeted tissues. What's more, this method is non-invasive and does not cause losses related to metabolism, as is the case with drugs passing through the digestive system [3]. The drug can be administered transdermal in the form of: ointments, creams, gels, microneedles or patches. Using hydrogels as carriers for cytostatic drugs can minimize side effects because the drugs are delivered directly to the target site. This can limit drug toxicity and reduce negative effects on other tissues and organs. In addition, hydrogels can hold cytostatic drugs at the site of administration for long periods of time, allowing the drug to act longer and more effectively on target cells or tissues [4,5].

Materials and Methods

The hydrogel materials were obtained by photopolymerization in a UV radiation field. The chosen method makes it possible to obtain a dressing material of any shape and size individually tailored to the needs of a particular patient. The hydrogel materials include a polymer base based on polyvinylpyrrolidone and protein carriers. The obtained materials will be subjected to detailed physicochemical analysis, including sorption capacity analysis, incubation studies in simulated body fluids, and spectroscopic analysis to determine the chemical structure of the obtained material.

Results and Discussion

The analyses carried out provided information on the sorptive capacity of the materials obtained. Importantly, the sorptive properties were confirmed and the obtained material is capable of absorbing wound exudates, which is particularly important considering its use as a dressing material. Spectroscopic analysis confirmed the obtaining of a properly cross-linked hydrogel structure, in turn, incubation studies showed no degradation of this material in fluids simulating the environment of the human body.

Conclusions

Hydrogel materials can be used as transdermal and controlled drug delivery systems for open wounds or diseases such as atopic dermatitis or skin cancer. Hydrogels can be designed to allow controlled release of cytostatic drugs. This allows drugs to be delivered in a gradual and controlled manner to target tissues or disease areas, which can improve therapeutic efficacy and minimize toxicity to healthy tissues. In addition, hydrogels can be customized in terms of their physicochemical properties, such as degree of swelling, viscosity, water absorption capacity, etc. This allows customization of the drug release profile and its interaction with target tissues. It is worth noting that the use of hydrogels as carriers for cytostatic drugs is being intensively studied, and many factors, such as the composition of the hydrogel, its morphology and release mode, can affect therapeutic efficacy. Further research and optimization of these systems may further develop this technology and improve cytostatic therapy.

Acknowledgments

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ZEOLITES AS ION-EXCHANGE FILLERS FOR DENTAL COMPOSITES WITH REMINERALIZING POTENTIAL

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Introduction

One of the top problems of restorative dentistry is the occurrence of secondary caries between the dental filling (restoration) and the remaining tooth tissues [1]. Its consequence is a decrease in filling durability, which has to be replaced then. Early caries that start with the demineralization of tooth tissues can be however reversed. Remineralization is the opposite process of demineralization. The demineralized structure of teeth is therefore rebuilt in the remineralization process in the presence of calcium, phosphate, and/or fluoride ions [2]. Placing the restoration with remineralizing potential into the cavity is one of the possible ways to prevent the development of caries. However, the current most common restorative materials with excellent aesthetic and mechanical properties, i.e. resin-based composites (RBC) are inactive in terms of remineralization. One of the possibilities is to make the RBC active by the addition of the filler able to release remineralizing ions, i.e. calcium, phosphate, or fluoride. Such composite in the aqueous environment of the oral cavity is to release ions that are able to rebuild the hydroxyapatite structure of hard tooth tissues. Ion exchange materials are a wide and promising group for such applications. It is assumed that thanks to the mechanism of ion exchange between the filler and saliva, the filling will not be significantly damaged over time while ensuring remineralization potential.

Materials and Methods

13X zeolite was synthesized according to the procedure described in [3]. After synthesis, 13 X was subjected to ion exchange to obtain its calcium form by soaking in a $CaCl_2$ solution. The additional hydroxyapatite layer was then formed at the surface. One part of the material was also silanized with MPS (3-(methacryloyloxy)propyl] trimethoxysilane.

All the obtained fillers were characterized in detail by means of X-ray Diffractometry (XRD), Scanning Electron Microscopy (SEM)/Energy Dispersive Spectroscopy (EDS), Transmission Electron Microscopy (TEM), Nitrogen Adsorption/Desorption Measurements, Thermogravimetric analysis (TGA) and Fourier-transform Infrared Spectroscopy (FT-IR).

Composites were prepared by mixing the obtained fillers with a methacrylic resins mixture (60% of Bis-GMA, i.e, bisphenol A glycerolate dimethacrylate, 40% of TEGDMA, i.e. triethylene glycol dimethacrylate) and polymerization initiators (CQ - capmhorquionone and EDMAB - ethyl 4-(dimethylamino)benzoate). After obtaining the homogenous pastes, the samples were placed in the forms (dimensions depended on the test type), which were protected on both sides with PET foil, and cured with the use of a dental LED curing lamp for 20 s.

Several parameters of the composites were examined:

- degree of conversion (DC) by means of FT-IR spectroscopy in ATR mode [4],
- depth of cure (DOC) according to ISO 4049 scratching test [5],
- mass stability after 14 days of incubation in saline at 36.6 °C by weighing method [6],
- compressive and flexural strength with the use of a universal testing machine [5,7],
- the ability to release calcium ions was examined with the use of Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) after 14 days of incubation in saline at 36.6 °C [3].

All the results were subjected to statistical analysis with the use of STATISTICA 13.0 software (TIBCO Software Inc.).

Results and Discussion

The structure of prepared fillers was confirmed by several methods.

The obtained composites show DC values between 77 and 86 %, which is far above than DC of typical RBC [8].

All studied composites show DOC above two limit values defined by ISO 4049, i.e. 1 mm (for opaque polymer-based dental materials), and 1.5 mm (for non-opaque materials) [5]. The application of silanized fillers resulted in composites cured to a greater depth [3].

Concerning the mass stability, of the obtained composites, the values of sorption and solubility are typical, i.e. few percent.

The typical chewing force is between 100 and 150 N [9]. The compressive strength of all examined composites exceeds these values, however, the values of flexural strength are insufficient.

All the composites show the remineralizing potential, understood as the ability to release calcium ions during incubation in saline. The higher the calcium content, the greater the amount of Ca^{2+} released during the experiment.

Conclusions

The application of zeolite ion exchange fillers in dental composites enables the preparation of active restorations that shows satisfactory functional properties with beneficial remineralizing potential.

Acknowledgments

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SILSESQUIOXANE MODIFIED HYDROXYAPATITE AS A FILLER IN DENTAL COMPOSITES

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Introduction

Hydroxyapatite (HA), the main inorganic component of bone and teeth [1] is a very interesting candidate as a filler for resin-based dental composites. HA is able to release calcium and phosphate ions, responsible for the remineralization process of tooth hard tissues [2]. Compounds from the group of polyhedral oligomeric silsesquioxanes are also of great interest. These organosilicon compounds, if contain methacrylic groups, can act as interesting additives to methacrylic resin-based composites (RBC). HA was successfully modified with polyhedral oligomeric silsesquioxane bearing methacrylic groups, namely heptaisobutyltri((meth-acryloxypropyl)dimethylsiloxy)heptasilsesquioxane (HIB-Met). HIB-Met was successfully synthesized. Eight different HA modification pathways were examined and evaluated. These modifications contributed to lowering the wettability of the HA surface, which is expected to increase the stability of the composite filled with this material in the aqueous solutions [3].

Materials and Methods

Fillers based on HA modified with HIB-Met were applied to obtain several different composites on the basis of methacrylic resins. Selection criteria for modified HAs included the economy of the modification procedures (reagents consumption, time), as well as their effectiveness in changing the wettability of the raw HA. RBC with unmodified HA, as well as HA silanized with MPS [((3-(methacryloyloxy)propyl) trimethoxysilane] (silanized according to [7] with slight modifications), were used as a reference. Fillers were mixed with Bis-GMA (bisphenol A glycerolate dimethacrylate) and TEGDMA (triethylene glycol dimethacrylate), as well as polymerization initiators, i.e. CQ (camphorquinone) and EDMAB (ethyl 4-(dimethylamino)benzoate).
After mixing the components together, the homogeneous pastes were placed in the forms, protected with the PET foil, and cured with the use of a dental LED curing lamp for 20 s.
The following physicochemical properties of the obtained composites were examined:

- degree of conversion using FT-IR spectroscopy in ATR mode [4],
- distribution of the constituents using Raman mapping,
- depth of cure (DOC) according to ISO 4049 scratching procedure [5],
- mass stability after 2 and 12 weeks of incubation in water at 36.6°C by weighing method described in ISO 4049 [5],
- compressive and flexural strength with the use of a universal testing machine [5-6],
- remineralizing potential, understood as the ability to release calcium ions during incubation in water at 36.6°C, with the use of a combined ion-selective electrode.

All the results were subjected to statistical analysis with the use of STATISTICA 13.0 software (TIBCO Software Inc.).

Results and Discussion

DC values were higher than for typical RBC, i.e. above 65% [8], showing no significant modification effect on the values of this parameter.
DOC of all examined RBC meets the ISO criterion for non-opaque polymer-based restorative materials, i.e. above 1.5 mm [5].
After 2 weeks of incubation in water, all composites showed sorption (SP) values higher than recommended by ISO 4049, i.e. above 40 μg/mm^3. In the case of composites with raw and silanized HA, also solubility (SL) values were higher than specified by ISO 4049, i.e. above 7.5 μg/mm^3 [5]. However, composites with HA modified with HIB-Met show lower solubility values, making them meet this criterion. As expected, Further incubation (12 weeks) of composites in water resulted in an increase in the SP and SL values. However, some interesting trends are observed. The evident effect of the HA modification is observed. SP of composites with modified fillers increases less than for the composite with raw HA. The difference in their SL is higher, but the total SL values are still lower than in the case of HA-filled composite.
No effect of the modification process on the mechanical properties of the composites is observed.
All examined composites are able to release calcium ions to the aqueous environment during the whole period of incubation (12 weeks). RBC with HA modified with HIB-Met show a lower amount of Ca^{2+} released from the beginning of the experiment, which can be attributed to their lower solubility. Additionally, in these cases, the concentration of Ca released faster reaches stabilization. This may result in the longer availability of calcium ions in places exposed to demineralization.

Conclusions

To conclude, the application of HIB-Met-modified HA fillers can be considered beneficial as it provides the advantages of HA filler with maintaining good physicochemical properties, improved stability in an aqueous environment, and most likely prolonged remineralizing potential.

Acknowledgments

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BIOMATERIALS USED FOR CLINICAL 3D BIOPRINTING OF BIONIC ORGANS WITH A FLOW SYSTEM: ASSESSMENT OF HEMOCOMPATIBILITY

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Introduction

Hemocompatibility of blood-biomaterial interactions is one of the most important criteria for their success in vivo applicability. Blood-biomaterial interaction can activate the coagulation cascade. Therefore, it is crucial for medical devices, as well as bioinks used for 3D bioprinting of bionic organs are hemocompatible. Biomaterials should allow direct contact with blood without a clotting effect. The regulation of blood coagulation is then a key issue in the development of biomaterials for medical applications.

Materials and Methods

The experiment was conducted on porcine whole blood without anticoagulant. The following biomaterials were used: methacrylate gelatin (GELMA), methacrylic hyaluronic acid (HAMA); methacrylate alginate (ALGMA), methacrylate chitosan (CHIMA), biomaterials based on extracellular matrix (dECM), pluronic (PLU), alginate (ALG), hyaluronic acid (HA) and positive control. The blood was added to the surface of the cross-linked biomaterials. The samples were incubated during: 1,3,5,8,10,12,15,20,25 and 30 minutes intervals. Then 1ml of water was added. Samples were shaken for 30sek at 300rpm. The absorbance was reading at 540nm. The higher the absorbance value, the higher the concentration of hemoglobin, which means less blood clotting on the surface of the biomaterial.

Results and Discussion

The analyzed biomaterials are significantly different in terms of hemocompatibility. PLU was characterized by immediate clot formation. At the 10th minute, the degree of hemolysis was also significantly lower in the case of ALG. Advantageously, the highest degree of hemolysis was found in dECM-based bioinks, regardless of its concentration. Thus, it was shown that dECM does not increase the coagulability of the tested blood.

Conclusions

Not every biomaterial due to the material composition despite preferential physicochemical properties and a beneficial effect on the viability and functionality of cells in vitro tests, will be suitable for bioprinting of bionic organs having direct contact with blood after implantation.

Conflicts of Interest

Michał Wszoła, Andrzej Berman and Marta Klak are the co-founders of Polbionica Ltd.

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MODIFICATION OF CALCIUM PHOSPHATES WITH VANADIUM AND MAGNESIUM IONS

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Introduction

Calcium phosphates have a special place in tissue engineering. Due to their biocompatibility and bioactivity, they exhibit the ability to form chemical bonds with natural bone tissue. By doping them with various ions, it is possible to improve their physicochemical and biological properties [1]. Mg ions play an important role in bone mineralisation by influencing the activity of osteoblasts and osteoclasts. They also have an indirect effect on mineral metabolism. Traces of it occur naturally in biological apatite in enamel, dentin and bone. Magnesium deficiency in the human body can lead to serious bone disorders such as bone growth arrest, reduced osteoblast and osteoclast activity, osteopenia or bone fragility [2]. Another interesting element for phosphate-calcium modification is vanadium. It promotes bone formation through a mitogenic effect on bone cells and, also, shows anticancer activity [3].

Materials and Methods

The present study describes the incorporation of magnesium and vanadium ions into synthetic hydroxyapatite obtained by wet precipitation. Ceramic mouldings were modified using in situ and sorption procedures. The phase composition and morphology of the resulting materials were determined by X-ray diffractometry, Fourier transform infrared spectroscopy and scanning electron microscopy equipped with energy-dispersive spectroscopy.

Results and Discussion

Differences were observed for the powders depending on the modifier chosen. Mg ions significantly affected the intensity of the FTIR absorption bands, as well as the signals observed by XRD. The results for V-enriched HAp are very similar to the spectrum for pure ceramics.
A difference in surface morphology as well as grain shape on SEM were observed (FIG. 1).

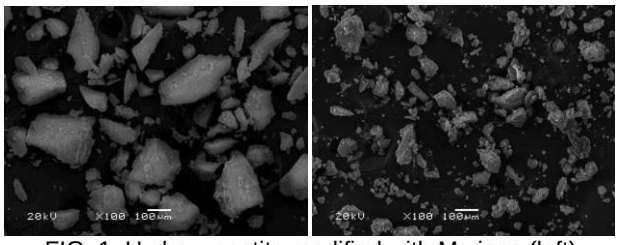

FIG. 1. Hydroxyapatite modified with Mg ions (left) and V ions (right).

Conclusions

It can be concluded that the selected powder modification method with the chosen ions is effective for Mg. No effect of V ions on the physicochemical properties of the ceramics was observed, however, more research is required.

Acknowledgments

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MAGNESIUM AND ITS ALLOYS FOR MEDICAL APPLICATIONS

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Abstract

Implants made from metallic materials are widely used in many areas of medicine, especially reconstructive medicine - skeletal, cardiovascular, respiratory or gastrointestinal surgery. Currently, titanium alloys (alloys with an α+β structure: Ti-6Al-4V, Ti-Al-Nb and single-phase β alloys), cobalt (cast Co-Cr-Mo, Co-Ni-Cr-Mo, Co-Cr-W-Ni alloys), stainless steels (mainly 316L steel) and shape memory alloys (Ni-Ti) are most commonly used in reconstructive medicine [101]. They have excellent mechanical properties and corrosion resistance, but also have drawbacks, including the release of toxic metal ions. Among biodegradable metal implants, magnesium, iron and zinc-based alloys (known as smart implants) have been extensively studied in recent years. In the case of magnesium and its alloys, most research has confirmed their desirable mechanical and biological properties (bioresorbability, biocompatibility and biodegradability). It is believed that magnesium and its alloys are the next generation of biomaterials and will have a major role in the revolution of orthopaedic, cardiovascular and dental applications. The development of biomaterials research is currently directed towards the transformation of their properties from bioinert to bioactive and their multifunctionality (antibacterial, anticancer) [102]. Consequently, controlling the corrosion behaviour of magnesium-based biomaterials is currently a major challenge.

Some results on mechanical, corrosion and biological properties, both in vitro and in vivo, have been reported in the literature, providing some indication of the potential for medical applications. Initially, studies were carried out on technical magnesium alloys (well known in the automotive and aerospace industries): AZ31, AZ91, LAE442, WE43, WE53. Unfortunately, magnesium alloys containing aluminium and heavy metals have been excluded as biomaterials due to the toxic effects of their additives on the human body. Tests are permitted for magnesium alloys containing biocompatible elements and/or small amounts of rare earth elements. These additives can be tolerated by the human body when present in appropriate concentrations. Tests have then been performed on magnesium alloys with crystalline and amorphous structures. The groups of magnesium alloys with crystalline structure are Mg-Ca, Mg-Zn, Mg-Mn, Mg-Si, Mg-Zr, Mg-Y. Magnesium alloys with amorphous structure have been obtained for phase equilibrium systems: Mg-Cu-Y(-Ag, -Pd, -Gd), Mg-Ni-Y(-Nd), Mg-Cu-Gd(-Zn, -Y), Mg-Zn-Ca. Some research has been devoted to the modification of the surface or chemical composition of magnesium alloys to improve their properties. Composite materials with appropriate bioactive reinforcements have also been developed as a solution to the problems of corrosion and low yield strength of pure Mg [103-106].

The paper is based on the results of a literature review carried out as part of the 6th edition of the Implementation Doctorate project. The problems addressed in this thesis relate to the development of technology for the production of magnesium-based biomaterials for medical applications. Bioresorbable magnesium alloys with controlled degradation times have the potential to revolutionise medicine. An ageing population will require new bioresorbable orthopaedic implants. The availability of such a biomaterial will reduce the cost of surgery, shorten the rehabilitation and recovery period and have a significant impact on the comfort of life for those requiring implants.

Acknowledgments

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BIOPOLYMERIC MATERIALS ENRICHED WITH SILK FIBROIN PROTEIN

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Introduction

Bone tissue regeneration is a complicated process that requires the interaction of various factors, including proteins, to effectively repair bone loss and damage. Fibrillar proteins play an important role in this process, as they are key components of the bone matrix with structural and regulatory functions. In the field of tissue engineering, the use of fibrillar proteins can contribute to the development of biomaterials as well as scaffolds that are more similar to natural tissue in bone and stimulate the regeneration process [1]. An example is collagen and elastin, both of which form the structure of the bone matrix, providing it with strength, elasticity and resilience [2]. In the context of biomaterials application, silk fibroin (FIG. 1) which demonstrates biocompatible and cytocompatible properties is of interest. Fibroin is formed by chains composed predominantly of a repeating sequence of amino acids like: (Gly-Ser-Gly-Ala-Gly-Ala) [3].

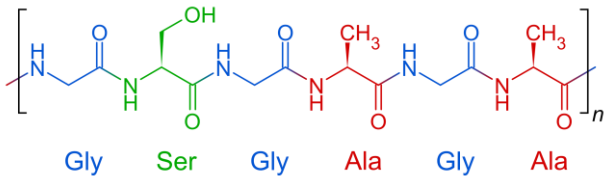

FIG. 1. Silk fibroin primary structure.

Materials and Methods

A composition of materials based on biopolymers, and biocompatible materials (including fibroin, chicken cartilage or PEGDA) was selected. Incubation studies were conducted in an artificial biological environment (PBS, Ringer's fluid) to evaluate the stability of the selected biopolymer composition under potential conditions of a living organism. Changes in pH values, conductivity, as well as sorption capacities were determined.

Results and Discussion

Small changes in pH values were observed, and conductivity increased with time. These changes, indicate that the material is not inert and interactions (like ion exchanges) occur at the material-liquid interface. Swelling abilities demonstrated a 3-fold increase in the weight of the liquid-weary biopolymer matrix. Even small swelling values of the material are a satisfactory result, as it indicates the possibility of using such a system in the delivery of active substances, since the drug is released as a result of swelling. This process is associated with the penetration of water molecules into the dry matrix of the dry material and subsequent hydration of the most polar hydrophilic groups. The swollen material exposes hydrophobic groups, which also begin to interact with liquid. As a result of the exposure of polar and hydrophobic sites, the polymer network has the ability to absorb additional liquid due to the osmotic pressure created.

Conclusions

It can be concluded that the developed materials, demonstrate stability in incubation fluids. They exhibit potential for use as a carrier of active substanation. The materials require further research and in the next steps, will be directed to modification with a bioactive ceramic phase.

Acknowledgments

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LIVER BIOCONSTRUCTS CREATED WITH INK-JET TECHNOLOGY FOR TESTING DRUG ACTIVITY AND TOXICITY

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Introduction
In the process of pharmacological research, a reproducible model representing the complex microenvironment of the human liver is still missing. Studies typically use 2D cultures, co-cultures, or spheroids that meet most testing needs but do not address the toxicity in the microenvironment of human tissues. This problem can be solved with bioprinted tissue models. The creation of such a bioconstruct is conditioned by the properties of the biomaterial, such as the stimulation of gas exchange and the distribution of nutrients, while maintaining the printability to create complex structures, such as hepatic lobules. We characterized the biocompatibility of biomaterials intended for the production of bioconstructs mimicking the microenvironment of human liver tissue.

Materials and Methods
We developed two formulations of biomaterials. The first consisted of methacrylates: gelatin and hyaluronic acid. The second was additionally enriched with decellularized extracellular matrix. Both variants contained the LAP photoinitiator. Hepatocyte and endothelial lines were suspended in culture medium and then mixed with the biomaterial. The hydrogel drops were created using ink-jet technology and cross-linked at 405 nm. The bioconstructs were cultured for 21 days on inserts. Microscopic imaging was performed using a live/dead staining, and the material was preserved for histological analysis

Results and Discussion
FDA/Pi staining showed high viability of the bioconstruct, staining more than 90% of viable cells with FDA, and only a few cells were stained with Pi. In addition, direct microscopic observation and histological examination showed an uniform distribution of the spherical cells with the ability to proliferate. Therefore, cells tend to form spheroids and migrate towards the surface of the bioconstruct.

Conclusions
Both bioinks showed biocompatibility with the liver cell line and therefore will be used for the bioprinting of liver tissue models with the flow system. The generated models will be applied in cytotoxicity and activity studies of biologically active substances.

Acknowledgments
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National Centre for Research and Development

Conflicts of Interest
Michal Wszoła and Marta Klak are the co-founders of Polbionica Ltd.

CHARACTERIZATION OF SYNTHETIC HYDROGEL USED IN ORTHOPEDIC APPLICATION

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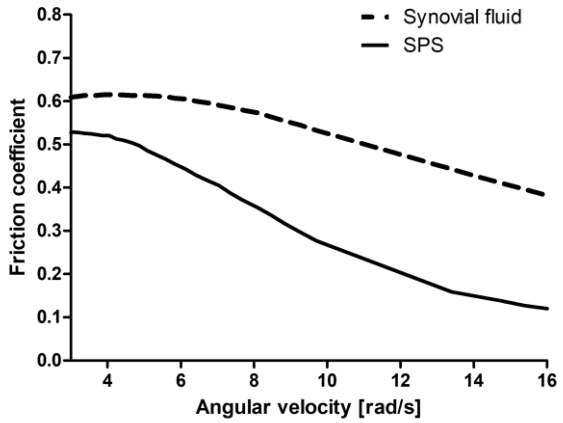
Introduction

The study presented in the following work aimed to evaluate the effectiveness and safety of a developed artificial synovial fluid for the treatment of pathologically diseased synovial fluid into the joint. The artificial synovial fluid is composed of a viscous and cohesive cross-linked polyacrylamide hydrogel, which is implanted into the joint. The material remains in contact with the synovial membrane of the joint, forming a layer that cushions it and encapsulates the cartilage, reducing friction between joint surfaces and relieving pain.
The study presented in the following work was designed to test the effectiveness of the proposed implant in lowering the coefficient of friction and to prove its safety.

Materials and Methods

The effect of selected manufactured prototypes on the friction coefficient of the metal ball-cartilage (bovine) system was investigated with usage of rheometer (Kinexus, Malvern Panalytical). The cytotoxicity test was carried using XTT cell viability assay (ATCC), on a stable WI-38 cell line.

Results and Discussion



The results were compared with the measurement for bovine synovial fluid (FIG. 1). The results in the range of 3-16 rad/s correspond to a frequency of 0.5 to 2.5 Hz, which is the frequency range of knee joint motion during walking (0.5 Hz) and running (2.5 Hz). The friction coefficient results obtained for the developed material were more favourable than those for the bovine synovial fluid. The artificial synovial fluid reduced the coefficient of friction in the tested system more effectively than the bovine synovial fluid. Moreover, studied prototypes did not show any cytotoxic potential during in vitro culturing with WI-38 cell line.

Conclusions

The described results demonstrate the effective reduction of the friction coefficient by the developed implant and prove its safety in vitro. The presented material, thanks to its reduction of the friction coefficient in the knee joint and its long-lasting pain-relieving effect, should improve the quality of life of patients with advanced osteoarthrosis.

Acknowledgments

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FULL INSULIN INDEPENDENCE AFTER TRANSPLANTATION OF 3D BIONIC PANCREAS TISSUE PETALS – LARGE ANIMALS RESULTS

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Introduction

Islets transplantation (ITx) is on the crossroad-in the US is treated as a drug, not a tissue transplantation, in Europe a few countries have a reimbursement for it. Search for a new implantation site didn't bring a breakthrough and still intra-portal ITx with its problems like instant blood-mediated inflammatory reaction(IBMIR) or stripping-off islets from extracellular matrix(ECM) after isolation is a gold standard. Nowadays a few attempts of stem-cell derived beta cells clusters (SCD-Beta) ITx has been shown with inconsistent results. Xeno-derived ITx are also ahead. It seems that ITx into portal vein SCD-Beta or Xeno-derived islets can face an exactly the same problems like standard donor derived ITx-IBMIR, lack of ECM and lack of insulin-independence. Additional safety reasons as such clusters in some extend will not be left in the liver make it difficult to monitor.
The aim of study was to show results of ITx in large animals of 3Dbioprinted bionic pancreatic tissue models as a further model to be used with SCD-Beta clusters or Xeno-derived islets.

Materials and Methods

12 pigs (weighing□30 kg) were divided into 4 groups: healthy pigs (n=3); animals after total pancreatectomy, treated with insulin(T1D;n=3); animals after total pancreatectomy and autotransplantation of pancreatic islets into the liver(Liver; n=3); animals after total pancreatectomy, and autotransplantation of 3Dbioprinted bionic pancreatic tissue petals with islets (3D-Petals; n=3). The effectiveness of the ITx was assessed by the daily insulin intake, C-peptide and glucose concentration up to 2 months post-ITx. 3Dbioprinted bionic pancreatic tissue models had 3x3x0.3cm in diameters and were transplanted between rectal muscle and peritoneum.

Results and Discussion

3D-Petals group reached full insulin-independence within 4-6 weeks post-ITx. Insulin intake in 2, 4, 6 weeks after ITx was 42; 16; 0% of T1D group requirement respectively ($P<0.01$). Insulin intake in the Liver group in 2, 4, 6 weeks after ITx was 24; 76; 69% of T1D requirement respectively ($P<0.01$). None of liver group achieved insulin-independence. Mean fasting C-peptide at 0, 2, 4, 6, weeks in healthy group was: 1.7, 1.51, 1.52, 1.21ng/mg vs. 1.5, 0, 0, 0 ng/ml in T1D group. C-peptide levels before and post-pancreatectomy and 2, 4, 6, 8 weeks post-ITx in 3D-Petals was 1.9; 0.0; 0.3; 0.16; 0.3; 0.32 ng/ml vs. 2.2; 0.0; 0.25; 0.1; 0.1; 0.1 ng/ml in the Liver respectively ($p<0.05$). The mean insulin intake in the T1D group was 5; 5.2; 6.4; 6.8 IU in 2, 4, 6, 8 weeks after pancreatectomy. In the LIVER group was 1.2; 3.95; 4.4; 5 IU vs. 2.1; 0.85; 0.0; 0.2 IU in the 3D-Petals in 2, 4, 6, 8 weeks after pancreatectomy and ITx.

Conclusions

3DBioprinted bionic pancreatic tissue petals transplantation achieved FULL insulin-independence in all animals which could be good prognostic before ITx of 3Dbioprinted pancreatic tissue with stem-cell derived islets.

Acknowledgments

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Conflict of Interest

Michal Wszoła, Andrzej Berman, and Marta Klak are the co-founders of Polbionica Ltd.

EXTRACELLULAR MATRIX AS A KEY COMPONENT IN THE PRODUCTION OF FUNCTIONAL AND PHYSIOLOGICALLY STABLE ARTIFICIAL PANCREATIC ISLETS USING THE INKJET METHOD

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Introduction
There are clinical trials report using stem cell-derived β-cells as an innovative and future-proof solution for the treatment of T1D. Stem cell-derived β-cells are expected to replace non-functioning pancreatic islets. However, in order to make this possible, it is necessary to create their 3D conformations, which has been proven by subsequent in vitro studies. However, apart from functionality, attention should be paid to the possibility of clinical use of beta cells. The process of transplanting claster β-cells even from 3D cultures, into the portal vein carries a high risk of damage and lack of functionality, as well as the risk of an undetermined final location of the cells. The aim of this experiment was to evaluate the survival and functionality of β-cells in artificial pancreatic islets, printed with inkjet method.

Materials and Methods
INS-1E cells were used in the study. Two bioinks were used as the encapsulation carrier: 2% HAMA + 20% GelMA (GROUP: H-G_INS); 2% HAMA + 20% GelMA + dECM (GROUP: ECM_INS). The control group was INS-1E in 2D culture. Cell functionality was assessed in the GSIS-test. In addition, FDA/PI vital staining was performed. One test sample contained 3.5 million β-cells.

Results and Discussion
During the 21-day observation, it was shown that the cells encapsulated by the inkjet method show practically 100% viability. However, they accounted for no more than 15% of the examined pool of cells. Cells suspended in the tested variants of hydrogels retained a stable structure and did not disintegrate. On the second day of the experiment, there was no difference in cell activity. Groups of encapsulated cells showed significantly improved functionality from day 7 onwards. Both groups showed over 30% higher functionality compared to the control group. On the 14th day of the experiment, cells suspended in bioink with dECM showed a definite superiority in response to the administered glucose. Compared to the control group, the increase was over 50% ($p=0.0005$), and with the H-G_INS over 30% ($p=0.0073$). Day 21 of the experiment also showed a functional advantage in the ECM_INS, almost 30% higher activity compared to the control group ($p=0.0040$).

Conclusions
dECM a 3D conformation of cells within a bioprinted islets is a key component for maintaining the proper functionality of insulin-secreting cells. In addition, the developed bioink composition and the method used enable the production of stable 3D structures that can be transplanted in a stable and safe manner without disintegrating in physiological temperature conditions.

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Conflicts of Interest
Michał Wszoła, Andrzej Berman and Marta Klak are the co-founders of Polbionica Ltd.

ENHANCING PRINTABILITY OF HYDROGELS BASED ON METHACRYLATED BIOPOLYMERS BY PRE-CROSSLINKING APPROACH

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Introduction

Methacrylated hyaluronic acid (HaMa) and methacrylated alginate (AlgMa) are widely used as additives to hydrogel-based bioinks. They exhibit very good biocompatible, biodegradable and non-toxic properties, thus supporting cell cultures. In addition, due to the presence of methacrylic groups in their chemical structure, after adding an appropriate photoinitiator, they have the ability to polymerization upon UV or visible light exposure. The limitation for these materials are inadequate visco-elastic properties, which play a significant role in extrusion-based printing. Fibers extruded from HaMa and AlgMa solutions, especially low-percentage ones, are too runny to obtain a high-resolution construct. In order to use the biological potential of hyaluronic acid and alginate, it is necessary to develop a method that improves the rheological and extrusion properties of materials.

Materials and Methods

Low-percentage solutions of methacrylates were used for the research, focusing on the solution of HaMa and AlgMa. Both variants contained the addition of photoinitiator (LAP), enabling for cross-linking at 405 nm. The materials were pre-crosslinked by exposing small amounts of solutions using visible light with a wavelength of 405 nm. Then, the prepared solutions were used in printability tests on an extrusion-based bioprinter. In addition, NMR measurements were performed to determine the dependence of cross-linking degree on exposure time.

Results and Discussion

It has been shown that the pre-crosslinking of low-percentage methacrylate solutions significantly impact on printability. The parameters used for initial cross-linking of the solutions resulted in only partial cross-linking of the material, which was shown by NMR tests. In printability tests, firm and homogeneous fibers were obtained, enabling the printing of high-resolution models.

Conclusions

The use of pre-crosslinking of low-percentage methacrylate solutions allows to improve their printability. The application of the presented method of preparing HaMa and AlgMa solutions gives new possibilities in using them for precise extrusion-based 3D printing.

Conflicts of Interest

Marta Klak is the co-founders of Polbionica sp. z o.o.

FISH COLLAGEN RADIATION STERILIZATION - IN AQUEOUS AND SOLID STATE

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Introduction

Fish collagen is considered to be one of the most promising materials to substitute bovine and porcine collagen. Apart from being fairly cheap and accessible source of animal protein it is a non-mammalian material provider, therefore it does not transmit most of the cow- and pig-originating diseases. Due to its biocompatibility and low cytotoxicity, collagen has found its use in a lot of branches of industry connected with beauty and health. Collagen can be used in pharmaceutical sector as injectable dispersion or microcapsules for drug delivery, ophthalmic sponges, as a supplement improving joint mobility or as artificial skin substitute for burn wound management.
Sterilisation is an important step of the preparation of materials and devices for the use in contact with living tissue of human body. Sterilisation of collagen has a main limitation that is a relatively low temperature of denaturation of the polymer between 29 and 37 °C. Therefore, the choice of the method should take into account the possible changes in the polymer matrix. We propose electron beam irradiation sterilization method. The aim of this work was to analyse the influence of the dose of electron beam irradiation on the chemical and physical structure of fish collagen.

Materials and Methods

Tropocollagen of silver carp (*Hypophthalmichthys molitrix*) was tested with and without a stabilizing agent of plant originated antibacterial peptides. The collagen gel (or solution) and solid lyophilizate, i.e. sponge, were irradiated by electron beam. Analysis of thermal and rheological properties revealed changes in triple helix content and the denaturation temperature, whereas SDS-PAGE electrophoresis demonstrated changes in molecular weight of denatured material.

Results and Discussion

Denaturation degree and its temperature is reduced for collagen with a stabilizer, but the collagen without the stabilizer denatured completely when treated in gel form with the dose of 25 kGy. Solid form of material is more stable the hydrated one. Electrophoresis examination revealed slight changes of molecular weight resulted from either degradation and crosslinking.

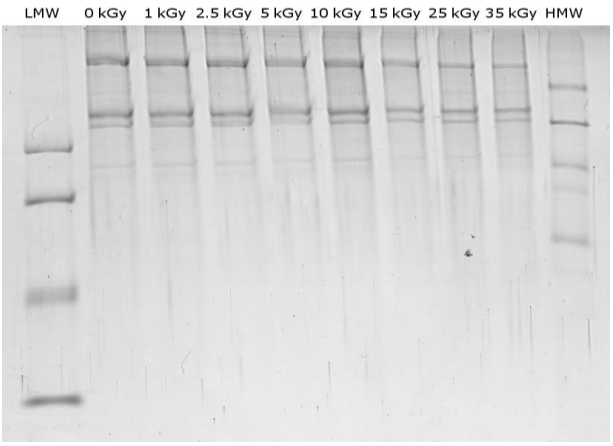

FIG. 1. Electrophoresis experiments of collagen without a stabiliser, data for gel (1.7%) and sponge irradiated at various doses by EB.

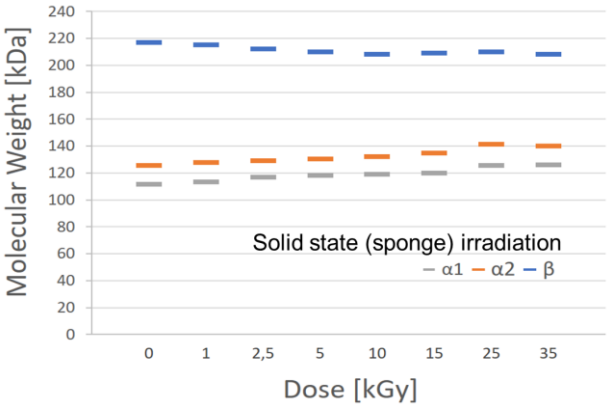

FIG. 2. Changes in molecular weight of α and β chains upon irradiation. SDS-PAGE electrophoresis.

Conclusions

It is anticipated that radiation method can be used for sterilization of collagen in a solid form.

Acknowledgments

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PHYSICOCHEMICAL CHARACTERISTICS OF THE PHOSPHATE CALCIUM CERAMICS OBTAINED

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Introduction

Calcium phosphate ceramics, including hydroxyapatite and brushite, are receiving increasing attention. One biomaterial that is finding use in hard tissue repair is brushite. It is a dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$ (DCPD), which is formed in phosphate deposits, soil and human dental calculi [1,2]. Several in vitro and in vivo studies suggest that brushite, along with other calcium phosphates, plays a huge role as an intermediate phase in the crystallisation of more stable hydroxyapatite [3,4]. Brushite is also currently being investigated as a cement for bone substitute materials. This material presents great potential for application in bone tissue regeneration medicine due to its properties.

Materials and Methods

In the present study, $CaHPO_4 \cdot 2H_2O$ was obtained using a wet method by synthesising calcium nitrate and sodium hydrogen phosphate at a pH of approximately 6.5. The resulting ceramics were then subjected to a freeze-drying process. Fourier-transform infrared spectroscopy (FT-IR) was performed and the resulting ceramics were subjected to X-ray diffraction studies. The Ca/P molar ratio of the synthesised brushite was also investigated. The calcium content of the tested ceramics was determined according to Polish standard PN-97/R-64803, and the phosphorus content was determined according to Polish standard PN-80/C-87015. In addition, the morphology of the obtained brushite was verified using Scanning Electron Microscopy (SEM).

Results and Discussion

An examination by Fourier-transform infrared spectroscopy (FT-IR) showed characteristic peaks for brushite, similarly, an X-ray diffraction (XRD) study demonstrated the presence of this phase. Furthermore, the study shows that the molar ratio of Ca/P is 1.0, a value that is in line with scientific reports of this material. Moreover, Using Scanning Electron Microscopy, the characteristic morphology of the obtained ceramics was demonstrated, which can be identified as a brushite phase.

Conclusions

In conclusion, based on the results obtained during the synthesis by wet precipitation, brushite was obtained. The results demonstrate the crystalline purity of the phase obtained and its calcium to phosphorus molar ratio coincides with published literature reports. The brushite phase obtained in this way, after biological analyses, could probably find its application in bone regeneration medicine.

Acknowledgments

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THE METHODS OF OBTAINING OF TITANIUM-HYDROXYAPATITE COMPOSITES

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Introduction

The lack of reactivity of metal-based implants results in cells not responding to them and instead surrounding them, hindering the growth of natural tissue. Although most metals exhibit this phenomenon, ceramics such as hydroxyapatite has a similar structure to bone and can promote the growth of natural tissues. By combining metals with a ceramics, a new biomaterial can be developed to possess the ideal mechanical strength and biological properties for bone implants and osteointegration [1]. Titanium-hydroxyapatite (Ti-HA) biocomposites are effective materials used in the field of biomedicine, specifically in implantology and bone reconstruction. the unique combination of titanium and hydroxyapatite not only provides high mechanical strength, but also improves biocompatibility and integration with biological tissues. In recent years, many studies have focused on developing new methods of obtaining these biocomposites to improve their quality and functionality. The aim of this article is to review various methods of obtaining Ti-HA biocomposites and to present their applications in the medical context.

Materials and Methods

Understanding and improving the processes of creating titanium - hydroxyapatite implants is crucial for the further development of these materials and improving therapeutic results in medical practice. This study collects and analyzes existing scientific publications on the methods of obtaining Ti-HA biocomposites. The studies included powder metallurgy, sintering electrochemical deposition, and binder jetting (FIG. 1). Principles of operation, process parameters and characteristics of the obtained materials for each of these methods were analysed [2].

Results and Discussion

The literature review showed that each of the methods has its own unique advantages and limitations that affect the structure, morphology and properties of Ti-HA biocomposites. Electrochemical deposition consists in the controlled deposition of a layer of hydroxyapatite on the titanium surface using electrochemical reactions [3]. For example, this method allows precise control of the chemical composition and thickness of the hydroxyapatite layer, while binder jetting offers speed of production and the ability to create complex geometries. What is more powder metallurgy ensures homogeneous dispersion of particles, sintering leads to durable connections, binder jetting enables precise creation of structures. Sintering is an effective method of producing titanium-hydroxyapatite materials, as it allows to obtain a controlled microstructure and interfacial connections, which are important for improving the biological and mechanical properties of implants. In addition, the sintering process can be used to form implants with complex shapes, ensuring precision and repeatability [4].

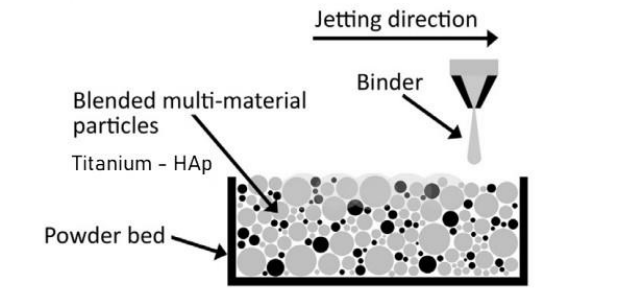

FIG. 1. The binder jetting process [5].

Conclusions

The choice of an appropriate method for obtaining Ti-HA biocomposites depends on the application requirements, such as structure, morphology, mechanical properties and bioactivity. Further research on the optimization of the production processes and the assessment of the impact of various parameters on the final properties of Ti-HA biocomposites are necessary in order to develop and improve these materials.

Acknowledgments

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BACTERIOCIDAL EFFECT OF CHEMICALLY MODIFIED CARBON NANOPARTICLES (CNPs)

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Introduction

In an era of increasing numbers of antibiotic-resistant pathogens, the search for new drugs and compounds with antimicrobial activity is very important. For several years now, nanoparticles have been a field for such exploration. The antibacterial activity of silver nanoparticles (AgNPs) [1] or gold nanoparticles (AuNPs) [2] has long been known, while less information is available on various forms of carbon nanoparticles (CNs) as antibacterial agents.

Materials and Methods

In this study, we evaluated the antibacterial activity of various allotropic nanoforms of carbon: nanodiamonds (NDs) and their modifications, as well as chemically functionalized carbon nanotubes (CNTs), fullerenes (Fu) and reduced graphene oxide (rGO). Antimicrobial activity was tested by three methods: agar gel diffusion method (modified Kirby-Bauer disc-diffusion method), in solutions and in layers using a modified method described in ISO 22196:2011 Measurement of antimicrobial activity on plastics and other non-porous surfaces. Nine types of nanodiamonds (ND1-ND9) modified in various ways were tested, including unmodified nanodiamonds as a reference control and modified carbon nanotubes, fullerenes and reduced graphene oxide. For testing, all nanoparticles were prepared as suspensions at concentrations of 0.1%, 0.01% and 0.001%, which were contacted with gram-negative (*Escherichia coli* ATCC 8739) and gram-positive (*Staphylococcus aureus* ATCC 6538) bacteria.

Results and Discussion

The results obtained by agar diffusion assay indicated the potential antimicrobial activity of ND6 and ND9, CNT, Fu and rGO. All nanoparticle suspensions were further tested in solution. They confirmed the antimicrobial activity of ND6 and ND9 and indicated the potential activity of ND5. Testing of nanotubes, fullerenes and the reduced form of graphene by these two methods, showed their high antimicrobial activity. Based on the results obtained from the two types of tests, carbon nanoparticle suspensions were selected for further testing conducted by a modified method based on ISO 22196:2011, in which nanoparticle suspensions were spread on the glass surface, dried and tested. After analyzing the results, pronounced antimicrobial activity was observed for all tested nanodiamonds (ND5, ND6 and ND9) as well as Fu and rGO, and slightly less for CNT.

Conclusions

The results obtained allow us to assume the possibility of a wide application of these nanoparticles not as drugs, but in the public space as additives to materials used to make everyday objects, such as handrails, handles, countertops or doors, objects that are ubiquitous and used by many people. Such applications of carbon nanoparticles with antimicrobial properties could be particularly attractive in clinics, hospitals, theaters, movie theaters offices or public transportation, where there is undoubtedly an increased amount of pathogens due to general accessibility.

Acknowledgments

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EFFECT OF SOLUTION COMPOSITION ON THE MORPHOLOGY AND PROPERTIES OF CHITOSAN COATINGS OBTAINED BY EPD

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Introduction

Biopolymers, including chitosan, are widely used for biomedical engineering applications. In view of its beneficial properties, such as being antibacterial, biocompatible and non-toxic, chitosan is a versatile natural polysaccharide used in many different fields. Multicomponent coatings with chitosan as the matrix are commonly produced using electrophoretic deposition (EPD) [1]. The preparation of suspensions for deposition involves the dissolution of chitosan in an aqueous acid solution. This is because chitosan does not dissolve in aqueous solutions, significantly limiting its use [2]. It dissolves only in water solutions of acids such as acetic, citric, formic, or hydrochloric acids, among others, while pH values are below 6.2 [2,3]. The aim of this work was to describe the influence of different acids on the structure and selected properties of chitosan coatings. In addition to the most commonly used acetic and citric acids, malic acid was selected as a novel possible solvent from the alpha-hydroxy acid group (AHA). It could potentially enhance the antimicrobial properties of chitosan coatings, due to its use in the food industry as a food preservative [4].

Materials and Methods

The chitosan coatings were deposited on commercially pure titanium Grade 1. To obtain a different chemical composition of the chitosan (2 g/l) solution, various acid - water mixtures were prepared: 4% of citric acid, 4% of malic acid and 1% of acetic acid. Chitosan was dissolved in acidic solutions and stirred for 72 h. Next, 50 vol. % of EtOH was added to each solution to prepare it for EPD and stirred for 15 min. Furthermore, the pH values of the prepared chitosan solutions were measured. The Ti substrates were ground using 800-grit sandpaper and ultrasonicated with acetone and EtOH for 5 min. The Ti sample was used as a cathode during EPD. The anode was an AISI316L steel plate. For AHA solutions (citric and malic acid) the voltage 5 V and time 6 min. were used during the process, while for acetic acid the voltage 10 V was applied for 6 min. The solutions were characterized by measuring the zeta potential and conductivity. Macroscopic observation of the coatings was performed with light macroscopy (LM). The morphology of the coatings was observed by scanning electron microscopy (SEM). FT-IR measurements of coatings in the middle infrared were performed to investigate their structure. The adhesion tape-test was carried out according to ASTM D3359-17. The wettability parameters, contact angle (CA) and surface free energy (SFE), were determined.

Results and Discussion

Macroscopically homogeneous coatings were obtained from a chitosan solution with acetic acid using a voltage of 10 V in a time of 6 min. To obtain similar quality coatings from chitosan solutions with alpha hydroxy acids, a lower voltage (5 V) was applied and the time was 6 min. Thus, the intensification of the water electrolysis process was inhibited. Based on the zeta potential and conductivity values of different solutions, it was found that the type of acid used does not influence the mechanism of chitosan molecule deposition on the cathode. The pH values of the AHA solutions containing chitosan were similar (2.6 for the solution with citric acid and 2.7 for the solution with malic acid) but lower than the pH value of the acetic acid-chitosan solution (4.3). Coatings obtained from solutions in which chitosan was dissolved in an acetic, malic and citric acid have shown a high adhesion strength, class 4B. Thus, the chemical structure of the acid used to enable the dissolution of chitosan does not strongly influence the adhesion strength of the coatings. FT-IR measurements provided an examination of the interactions between the molecules of acids and chitosan. Acetic acid only causes, to a minor extent, protonation of the free amino groups of chitosan. The addition of citric or malic acid, which are polycarboxylic acids, results in the ionic cross-linking of chitosan molecules. The process is based on the interaction of carboxyl and amine groups, therefore it is more effective with a higher degree of deacetylation. Tricarboxylic citric acid could provide more complex ionic cross-linking, but is limited by steric hindrance and occurs similarly as for dicarboxylic malic acid. Coatings obtained from chitosan solutions with AHA on Ti substrates have shown a slightly higher hydrophilic character (CA=48±4°) than the substrate (CA=56±2°). In comparison, the coatings obtained from a chitosan solution with acetic acid had a higher CA value with water (CA=84±1°) than the substrate.

Summary

Chitosan coatings were electrophoretically deposited on Ti substrates from solutions with different acids. Macroscopically homogeneous coatings were obtained using 4% citric acid or 4% malic acid solutions at 5 V for 6 min and 1% acetic acid solutions at 10 V for 6 min. The chemical structure of the acid used to dissolve chitosan had no significant effect on the adhesion of the coatings to the substrate. All coatings showed a similar high adhesion strength to the Ti substrate, class 4B. The main noticeable difference was the wettability values of the prepared coatings, which differed within the group of acid used as a solvent. Chitosan coatings have shown wettability different from Ti substrates due to the acid used in the solution. However, the values of CA were similar in the AHA group. Further characterization, including a microbiological study of the coatings, is in progress.

Acknowledgments

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COMPOSITE BASED ON POLYVINYL ALCOHOL, CHITOSAN, AND CURCUMIN FOR WOUND HEALING APPLICATIONS

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Introduction

Chitosan is a polysaccharide, and it is derived from chitin. Dermal wounds are considered to be complex; the major reason of dermal wounds complexity is the disruption of the normal tissues of the skin. Several biopolymers can be used for fabrication of wound patches. Chitosan is one of them. It is polysaccharide derived from chitin. Chitosan usually is applied in the form of the films or gels. Advantage of applying chitosan in wound healing is that physical and chemical properties of chitosan can be modified to meet the wound healing conditions. The wound dressings of chitosan without encapsulated fibrous reported with minimum side effects, and it presented anti-bacterial properties [1]. Polyvinyl alcohol is also applied in wound healing due to excellent mechanical strength and gas permeability [2]. Curcumin is a natural compound, and its use in wound healing is due to anti-bacterial, anti-cancer, and anti-oxidative properties [3]. In this work the composite based on polyvinyl alcohol, curcumin, and chitosan have been researched for potential wound healing applications.

Materials and Methods

Polyvinyl alcohol (363065, CAS:9002-89-2, MW: 146,000-186,000) obtained from Sigma-Aldrich, Germany. Curcumin (GP8291, CAS: 458-37-7, MW: 368.39) from Glentham Life Sciences, United Kingdom. Ethanol (Cat. 32294, CAS: 64-17-5, MW: 46.07, 96%) from Honeywell, Acetic acid (CAS:64-19-7, MW: 60.05, 99.9%) from STANLAB, Lublin, Poland have been used.
Solutions of polyvinyl alcohol (PVA) 5% (w/v) in water, chitosan 2%(w/v) in acetic acid, and curcumin 1mg in 5 ml of ethanol have been prepared. Polyvinyl alcohol and chitosan polymeric blend has been prepared and 2% curcumin have been added. Polymeric films of polyvinyl alcohol, chitosan, and of polymeric blend (PVA5%, chitosan 2% and curcumin 2%) have been prepared by the solvent casting technique (solvent evaporation technique). Mechanical properties of polymeric films of PVA 5%, chitosan 2%, PVA 5%: curcumin 2%, PVA 5%: chitosan 2% : curcumin 2% have been analysed by Zwick and Roell 0.5 mechanical properties testing machine. Fourier Transform Infrared (FTIR) spectrum of polymeric films and curcumin powder have been analyzed with Nicolet iS10 equipped with ATR device. Contact angle measurements have been made with drop shape analyzer (DSA) 10, KRÜSS, Germany to evaluate the hydrophobic and hydrophilic properties.

Results and Discussion

Chitosan and polyvinyl alcohol were found to be compatible and the blend has shown excellent mechanical properties. Addition of curcumin proved to enhance the biological activities. FTIR spectrum has shown the presence of materials in the polymeric blend. FTIR spectrum has been presented in the FIG. 1.

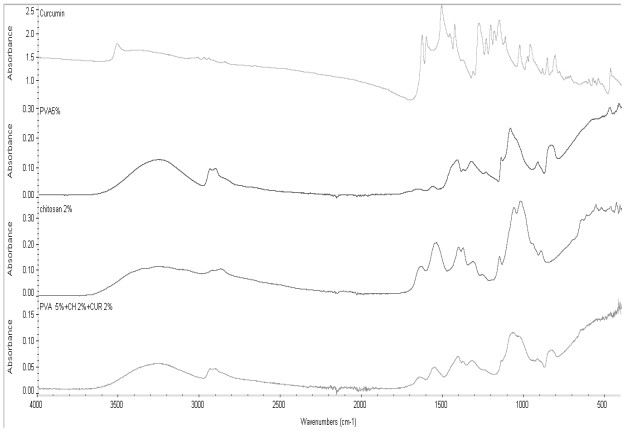

FIG. 1. FTIR Spectrum of films obtained from PVA 5%, Chitosan 2%, Curcumin powder, and polymeric blend composed of PVA5%, chitosan 2%, and curcumin 2%.

Contact angle for the obtained composite films have been measured, and surface energy calculations have been carried out. Surface properties are crucial for application as wound healing materials.

Conclusions

Polymeric blends prepared in this research presented excellent mechanical strength. In the polymeric blend of polyvinyl alcohol 5% and chitosan 2% curcumin can be incorporated. FTIR spectrum of polymeric blend confirmed the presence of curcumin. Contact angle has been monitored, and surface energy calculations have been calculated accordingly. It has been noted that curcumin addition to polymer blend led to the modification of the surface properties.

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BIOPOLYMER BLENDS FOR TOPICAL APPLICATION ON THE SKIN: PREPARATION AND PROPERTIES

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Introduction

Biomaterials based on polymer blends are increasingly being investigated for their application potential. Blends can be prepared by mixing two or more polymers [1]. Such a procedure affects the structural and physical properties of the polymers and leads to a material with new desired properties that are superior to any one of the component polymers [2]. Two ways of preparing blends can be distinguished: blending in molten state and dissolution in the same solvent [1]. A second method was used in this study.

Chitosan (CS) is one of the popular biopolymers for topical applications on the skin. It is a linear polymer, a derivative of chitin. It is non-toxic, biocompatible, and has anti-oxidation potential [3]. There are many reports in the literature of blends of chitosan with collagen, cellulose, gelatine, hyaluronic acid or polyvinyl alcohol [4]. A less well-known combination is the blending of chitosan and glucomannan, particularly for biomedical and cosmetic applications.

Glucomannan (GM) is also a linear polysaccharide, consisting of D-glucose and D-mannose units in a molar ratio of 1:1.6. This biopolymer is extracted from tuberous root of konjac [5]. The combination of chitosan and glucomannan in the form of a blend can create a material with new properties.

Materials and Methods

Chitosan (low molecular weight) and konjac glukomannan have been purchased from the POL-AURA company (Poland).

Initial polymer solutions were prepared in 0.1M acetic acid (CS 2% [m/v]; GM 0.5% [m/v]). Blends were prepared by mixing the starting solutions in ratios of 80:20, 50:50 and 20:80 for chitosan and glucomannan, respectively. The solutions were stirred for 24 hours and then poured 25g onto 10 x 10 cm polystyrene plates. the initial solutions were also poured out. The samples were dried at 37°C until completely dry. After this time, the resulting films were pulled off and the analyses were performed.

The morphology of the samples was studied using a scanning electron microscope (SEM) (LEO Electron Microscopy Ltd., Cambridge, UK).

Mechanical properties were measured by the use of a mechanical testing machine (Z.05, Zwick and Roell, Ulm, Germany). Samples were cut in the shape of paddles (width 4 mm in the center). Testing program parameters were fixed as follows: the speed starting position was 50 mm/min, the speed of the initial force was 5 mm/min, and the initial force was 0.1 MPa. Data were collected using the TestXpert II 2017 program, and results were presented as average values with standard deviation.

ATR-FTIR spectra of each sample were obtained (the range of 400–4000 cm^{-1}, absorption mode at 4 cm^{-1} intervals, 64 scans). To record spectra, a Nicolet iS10 spectrophotometer with an ATR accessory and diamond crystal (Thermo Fisher Scientific, Waltham, MA, USA) was used. To process data, OMNIC software (Thermo Fisher Scientific, Waltham, MA, USA) was used.

Results and Discussion

Scanning electron microscope (SEM)

Scanning electron microscope allows observing the structure of obtained films (FIG. 1). Chitosan has the smoothest surface structure. The higher the content of glucomannan in the blend, the more uneven the surface of the film.

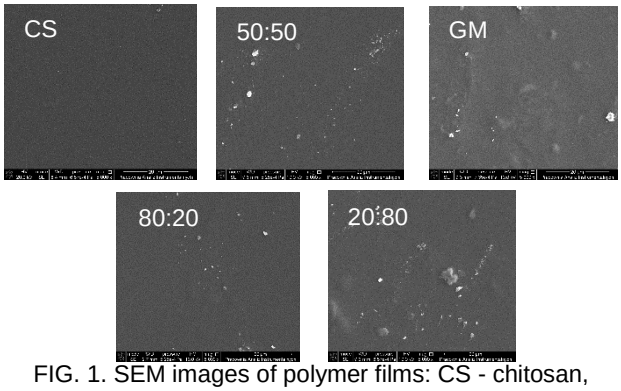

FIG. 1. SEM images of polymer films: CS - chitosan, GM - glucomannan, 50:50, 80:20, 20:80 - chitosan/glucomannan blend ratios respectively (magnification 5000x).

Mechanical properties

Mechanical properties of the biopolymeric films were determined using a tensile test. The lowest Young's Modulus values (FIG. 2) were observed for the CS sample. The addition of glucomannan increases the values of this parameter. The higher the content of GM in the blends, the higher the Young's Modulus, which correlates with the brittleness of the material. The highest value was obtained for the 20:80 (CS:GM) blend.

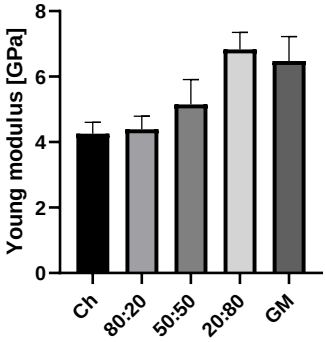

FIG. 2. Young's modulus values for individual samples; for $p < 0.05$, no statistically significant differences were observed.

ATR-FTIR Spectroscopy

Characteristic FTIR spectra were obtained for the initial polymers, confirming their chemical structure. Individual bands in the blends occur at the same wavelengths as in the starting polymers.

Conclusions

The results showed that it was possible to obtain blends of chitosan and glucomannan in a simple manner. The films obtained from the blends could be easily removed from the polystyrene plates and samples could be easily cut out for analysis. The results of mechanical measurements are promising in terms of applicability on the surface of the skin.

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THE INFLUENCE OF FINISHING TECHNIQUE ON PORCELAIN VENEERS WETTABILITY

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Introduction

An appropriate veneer materials are required to fulfil demands for good functional and highly aesthetic restoration. Dental ceramic is used widely for veneers as it mimics the natural tooth properties such as light transmission, colour reproduction and texture. Several finishing and polishing procedures are used to achieve a smooth, shiny surface and avoid clinical problems, e.g. increased dental plaque retention and periodontal tissue inflammation, increased staining and lower resistance to crack propagation [1-4].
The aim of the work was to estimate the influence of finishing techniques on wettability and surface free energy of ceramic veneers.

Materials and Methods

Samples were prepared form two types of ceramic: (1) natural VitaVM7 (Zahnfabrik H. Rauter, Germany) and (2) synthetic Vintage MP (Shofu, Japan). Samples were prepared according to the manufacturers' instructions in the shape of inserts with dimensions 10,0x10,0x2,0 mm. The samples surfaces were submitted to the following surface treatment: polishing (rubber wheels) (p); polishing with diamond paste (pp); glazing (g); glazing with glaze (gg).
Contact angle was measured using the sessile drop method on the optical goniometer (Advex Instrument, Czech Republic). Three probe liquids were used: distilled water (Biomus, Poland), diiodomethane (Merck, Poland) and glycerol (Chempur, Poland). The drop volume was 0,5 µl. The final contact angle, used for analysis, was an average of ten values. The surface free energy (SFE, γ_s) was calculated according to two approaches: Owens-Wendt (OW) and van Oss-Chaudhury-Good (OCG).

Results and Discussion

Two types of tested ceramics showed differences in wettability. Both ceramics had hydrophilic surface character. The glazing (g) and glazing with glaze (gg) ceramic surfaces showed higher wettability compared to polished surfaces (FIG.1). Differences between two types of ceramics are not statistically significant. The values of SFE are in the range from 58,46 to 67,77 [mJ/m^2] according to OW method, and from 38,00 to 56,35 [mJ/m^2] according to OCG method (TABLE 1 and 2).

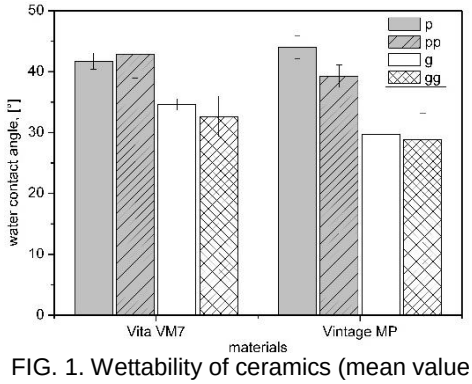

FIG. 1. Wettability of ceramics (mean value and standard deviation).

TABLE 1. SFE and its components (polar (γ^p) and nonpolar (γ^{np})) calculated acc. OW methods [mJ/m^2] (mean value and standard deviation).

Material		γ_s	γ^p	γ^{np}
1	p	60,61 (1,55)	25,03 (1,06)	35,58 (2,20)
	pp	58,46 (1,72)	26,42 (3,91)	32,04 (3,03)
	g	64,94 (0,74)	28,70 (0,77)	36,24 (1,11)
	gg	66,27 (1,20)	33,18 (1,10)	33,09 (0,62)
2	p	59,58 (1,25)	23,29 (1,40)	36,30 (1,47)
	pp	60,75 (1,32)	28,26 (0,98)	32,50 (0,90)
	g	64,76 (1,85)	31,53 (1,74)	33,23 (0,86)
	gg	67,77 (2,42)	31,76 (2,61)	36,01 (2,82)

TABLE 2. SFE its components (polar (γ^p) and nonpolar (γ^{np})) calculated acc. OCG methods [mJ/m^2] (mean value and standard deviation).

Material		γ_s	γ^p	γ^{np}
1	p	56,35 (1,09)	20,78 (1,19)	35,58 (2,20)
	pp	38,00 (6,35)	5,96 (5,74)	32,04 (3,03)
	g	49,64 (3,69)	13,40 (4,31)	36,24 (1,11)
	gg	36,65 (1,40)	3,56 (1,65)	33,09 (0,62)
2	p	56,21 (1,65)	19,91 (1,93)	36,30 (1,47)
	pp	39,83 (3,30)	7,33 (3,58)	32,50 (0,90)
	g	52,52 (1,92)	19,29 (2,20)	33,23 (0,86)
	gg	54,47 (2,10)	18,46 (2,65)	36,01 (2,82)

Values of SFE energy obtained for different surface finishing techniques are visible only for OCG approach (combination of three probe liquids). The lower SFE value can be observed for polishing ceramic with diamond paste. For both approaches, the dispersive component has the predominant share in the SFE value which can show a higher adhesive affinity for non-polar substances.

Conclusions

Comparison of surface finishing techniques for tested ceramics showed that after glazing the surface is more hydrophilic showing higher values of surface free energy compared to polishing technique. It can influence on bacterial adhesion as it is more likely on hydrophilic surfaces [5].

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COMPARISON OF SURFACE ENERGY OF DENTAL FLOW COMPOSITES

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Introduction

Flowable resin-based composites are restorative polymeric materials of low viscosity, through decreasing the filler share (37–53% vol.) and/or by intergrading less viscous monomers [1]. Lower viscosity allows their extrusion out of the syringe tip into intricate cavities and good adaptation to the cavity walls. Flowable system ensure better curing in depth, which can fill up to 5 mm at once satisfactorily as compared to conventional composites [2, 3]. An important requirement to establish adhesion and bonding of composites to dental tissue are surface properties e.g. optimal wettability of the surface.
The aim of the research was to compere surface properties of two types of flowable dental composites: the dentin shade dedicated for aesthetic results (depth of cure 2 mm) and the bulk shade for deep cavities (depth of cure 5,5 mm).

Materials and Methods

Samples were prepared form the flowable dental composites: everX Flow (GC Corporation, Japan) occurring in two shades: dentin (D) and bulk (B). Tested materials are composed of polymer resin (Bis-MEPP, TEGDMA, Bis-MEPP), glass fibre (25% vol.) and particulate filler (42-52% vol., barium glass). Samples were prepared according to the manufacturers' instructions in the shape of cuboids with dimensions 10,0x10,0x15,0 mm. Contact angle was measured using the sessile drop method on the optical goniometer (Advex Instrument, Czech Republic). Three liquids with a well-known value of surface tension were used in the tests: diiodomethane (Merck, Poland), glycerol (Chempur, Poland), distilled water (Biomus, Poland). The drop volume was 0,5 µl. The measurements of each surface and each liquid were repeated 10 times. The final contact angle, used for analysis, was an average of ten values. The surface free energy (SFE, γ_S) was calculated according to following approaches, differing in the number of probe liquids (nL): Kwok-Neumann (KN, 1L), Li-Neumann (LN, 1L), Owens-Wendt (OW, 2L), Wu (W, 2L) and van Oss-Chaudhury-Good (OCG, 3L) [4]. The calculation methods of SFE were used for estimating the cohesive energy density (e_c (MJm^{-3}) of materials and solubility parameter which is defined as the square root of cohesive energy density [6].

Results and Discussion

Two types of tested flow composite showed differences in wettability. Both materials have hydrophilic character but dentin shade dedicated for deep cavities had higher value of the contact angle (FIG. 1). Comparison of several approaches used in SFE calculations showed differences between tested materials and also influence of SFE model on obtained values (TABLE 1). The values of γ_S obtained from OW and W models are similar to values referred in [5] for dental composites (from 48,4 to 50,7 [mJm^{-2}]). The cohesion energy density values [MJm^3] range from 219,2±99,8 to 758,0±150,2 for dentin shade and 283.7±97,8 to 616,1±22,7 for bulk shade (TABLE 2). The solubility values for dentin and bulk shade range from 16,8±3,2 to 24,8±2,9 [$(MPa)^{0,5}$] and are similar as referred for PMMA materials (19-26) [6].

TABLE 1. Comparison of γ_S [mJm^{-2}] obtained by different approaches (mean values and standard deviation).

Mat.	KN (1L)	LN (1L)	OW (2L)	W (2L)	OCG (3L)
D	32,50 (1,43)	32,90 (1,39)	42,73 (1,56)	49,19 (1,60)	62,10 (8,45)
B	38,08 (2,14)	38,40 (1,16)	47,70 (1,28)	54,29 (1,33)	48,09 (3,57)

TABLE 2. Comparison of e_C [MJm^{-3}] obtained based on different model od SFE (mean values and standard deviation).

Mat.	KN	LN	OW	W	OCG
D	292 (101,3)	219,2 (99,8)	430,2 (23,5)	513,4 (26,0)	758,2 (150,2)
B	363,8 (64,0)	283,7 (97,8)	507,3 (20,4)	616,1 (22,7)	514,4 (57,4)

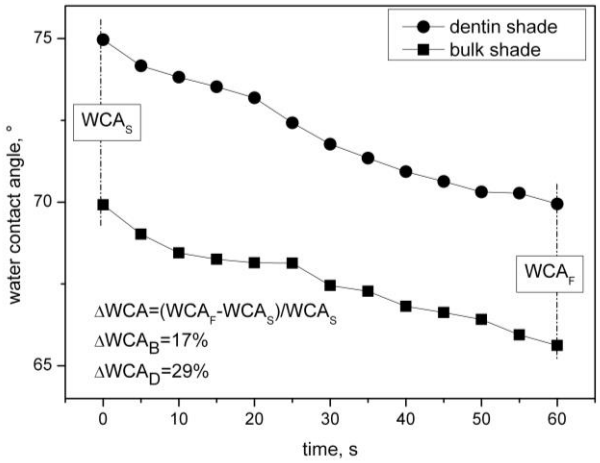

FIG. 1. Water contact angle during 60 s for the everX.

Conclusions

The energy cohesion density can be used to predict the extent of environmental stress cracking. Differences in SFE between tested types of flowable composite influence on its binding capacity to dental tissue. Comparison of several approaches of SFE energy calculation showed importance of choosing probe liquids and related to them information about surface effect.

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NOVEL FUNCTIONAL BONE CEMENT BASED ON MAGNESIUM PHOSPHATE AND ALGINATE HYDROGEL DEDICATED TO THE MINIMALLY INVASIVE BONE DEFECTS TREATMENT

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Introduction

An advantageous functional feature of modern biomaterials is injectability, which enables their use in minimally invasive surgery. A specific group of self-setting injectable materials, called bone cements, is widely used in medical treatment, and two kinds are routinely commercially applicable: ceramic (based on calcium phosphates) and polymer (mainly poly(methyl methacrylate)) [1]. Magnesium phosphate cement (MPC) has recently become an interesting alternative to the mainly used ceramic cements – i.e., based on calcium phosphates. MPC is characterized by more efficient resorption in the human body, more favorable (initial) mechanical strengths, and shorter setting time [2]. This work evaluated the influence of alginate hydrogel addition to magnesium phosphate cement mainly to improve its functional properties.

Materials and Methods

The ceramic cement was produced by reacting magnesium oxide (Fisher Chemical, USA) with potassium dihydrogen phosphate (Chempur, PL) in a 4:1 molar Mg-P ratio, in a water environment (with various powder-liquid ratios). Modified cements were obtained by using aqueous solutions of sodium alginate (SA; Chemat, PL) as the liquid component of cement. Further, polymer hydrogel was cross-linking in a delayed reaction [3]. After mixing the cement components (powder and liquid), the resulting paste was transferred to special molds and stored for 24 h at 37 °C and > 90% humidity (water bath). The cement powder was mixed with water and treated identically to the tested cements as a reference. The following research was carried out: setting time (Vicat apparatus), setting temperature (thermocouple), microstructure (SEM), chemical and phase composition (XRD diffractometry and FTIR spectroscopy), compression strength (Universal testing machine), injectability (qualitative assessment), biodegradation (immersion in the PBS solution for a one month) and cytocompatibility tests on human osteoblasts hFOB 1.19 (ATCC; with MTT assay and agar test).

Results and Discussion

In this research, a novel biocomposite cement based on MPC and SA was successfully developed. In each group of tested cements, the formation of magnesium phosphate crystals was confirmed by SEM microscopy and XRD analysis. Their different sizes, growth, and degree of unreacted MgO were observed for various technological parameters. The amorphous phase of the alginate hydrogel was also found, and its cross-linking process was confirmed by FTIR spectrometry. All modified cements have a setting time shorter than 15 minutes, which is suitable for medical applications [4]. In the case of the reaction temperature, which should not exceed 50°C [1], some modified cements showed its reduction. In the injectability tests, poor cohesion was found only for the MPC control, while improvement in cohesion and injectability was observed in all tested cements. Most obtained groups showed slightly reduced mechanical properties (compressive strength and Young's modulus). However, the decrease in elastic modulus suggests the material is less brittle [5]. All specimens degraded in conditions simulating the human organism (mass loss ~3% / month of incubation), while in a short period (up to 5 days), they remained biostable. Osteoblasts cultured on the surface of cements showed a decrease in their survival (% of MPC control, MTT assay) – and this could be related to the disturbance of the ion balance in the medium [6], hence an agar test was also performed to confirm cytocompatibility of developed cements.

Conclusions

- The use of alginate hydrogel allows to reduce in the setting time and setting temperature of MPC cement.
- An improvement in all modified cement groups of injectability and paste's cohesion was confirmed.
- The polymer addition had a negative effect on the cement's mechanical strength.
- Both setting reactions (ceramic and cross-linking) were evaluated as effective.
- Cements remained biostable in the short incubation period, while appropriately degraded in the longer time.
- Specimens created a specific hydroxyapatite on their surface, which disturbs the ionic balance of the culture medium and then had a negative effect on cellular response in *in vitro* tests.

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Funding

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EXPERIMENTAL EVALUATION OF ND: YAG LASER PARAMETERS AND SAMPLE PREPARATION METHODS FOR AISI316L STEEL

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Introduction

Material engraving with a laser beam can be used to mark the product, obtain specific visual and decorative effects or modify the surface layer of a given material. Laser ablation involves the removal of material through the process of evaporation of the material from the surface. Laser ablation is most often performed with short-pulse lasers, but depending on the power of the laser beam, the process can also be performed using lasers for continuous operation. [1]. Conducting laser modification to the surface layer changes the composition of the alloy and gives it different microstructural features, but only on the surface of the material, which often results in improved resistance to corrosion and fatigue cracks formation [2, 3].

When using a laser beam to modify the surface layer, special attention should be paid to the surface preparation process [4, 5]. In the case of engraving, maximizing the efficiency and repeatability of the process is the key to obtaining the desired properties. Engraving a shiny metal surface can lead to laser beam dispersion and energy loss. Some materials require special preparation and surface darkening in order to be effectively engraved [6]. Stainless steel AISI 316 is the second most commonly used stainless steel grade in the industry. The 316L version is a low carbon variant of the 316 alloy, which makes the 316L stainless steel suitable for welding, which is especially important in the case of laser processing. [7, 8].

Materials and Methods

The research stand consists of a TRUMPF TruLaser Station 5004 device with a built-in touch screen and a manual control panel and an adjacent computer stand with dedicated TruControl 1000 software, version 1.92.0, provided by the manufacturer. The view of the test stand is shown in FIG. 4.3.1.1. At the back of the station there is a cylinder with a dedicated shielding gas. For this device, Argon 4.8 according to ISO 14175-I1-Ar was used. The station is also equipped with an additional camera that allows you to preview the positioning of the head and the laser modification process on a separate monitor.

The dedicated TruControl 1000 software allows the user to design a modification path by developing a CNC program based on a list of available command codes, and then selecting laser processing parameters. The system allows changing three key parameters of the laser (power, frequency, focusing) in various ranges, as listed:
Power 300 - 6000 W;
Frequency 0.2 - 833 Hz;
Focus 0.4 - 1.85 mm.

The *focus* parameter approximately corresponds to the diameter of the laser spot. Built-in security systems prevent the setting of dangerous parameters that threaten the user's safety or threaten to damage the station. In some cases, this results in automatic changes of some parameters or blockage of the laser beam preventing the start of the process.

Results and Discussion

Using the CNC program, a series of laser modifications of AISI 316L steel samples with dimensions of 50x50x5 mm was made. A simple pattern was chosen in order to focus on the parameters and sample preparation and not on coding. The laser beam goes as follows - straight line from left to right, then move up and straight line again, to the left, move up, then loop the sequence as many times as sample size allows. Laser parameters were selected and changed based on current observations. The constant parameters were the pulse time, which was 1 ms, and the laser head speed, which was set to 2 m/s.
Lack of sample preparation emerged problems such as: sample deformation, sparks formation of sparks which in many cases led to destruction of the already applied track, irregular shape of individual dimples and local material burn-throughs.

Conclusions

The best effect was obtained by laser power range 4800 - 6000 W applied to samples previously grinded with sandpaper with a gradation of 800 using a manual two-disc Saphir 330 sander and polisher, ATM GmbH, German production, and then etching in a mixture of concentrated (65%) hydrochloric and nitric acid in a volume ratio of 3:1, and the subsequent darkening of the surface of the sample with graphite or black alcohol-based ink from the popular *permanent marker*. The samples prepared in this way were subject to much less deformation during the laser process. With properly selected laser parameters, the problem of deformation was almost completely eliminated. The darkening of the surface also prevented sparks formation.

Acknowledgments

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DEVELOPMENT OF DEGRADABLE RODS BASED ON COPOLYMERS FOR PROLONGED RELEASE OF ARIPIPRAZOLE

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Introduction

Aripiprazole (ARP) is a neuroleptic used mainly in the therapy of mental illness [1]. Due to poor adherence during therapy by oral formulations, ARP long-acting injections (ALAIs) have begun to develop [2]. Currently, there are only two medicinal products of this type on the market. However, their use is associated with several inconveniences, *e.g.* (i) the need for oral supplementation for two weeks; (ii) no possibility of removal if side effects occur; (iii) the occurrence of pain with intramuscular injections; and (iv) frequent outpatient care. This study aimed to develop ALAIs in the form of rods based on poly(D,L-lactide-*co*-glycolide) (D,L-PLGA) copolymers with different compositions.

Materials and Methods

D,L-PLGA (50:50) and D,L-PLGA (70:30) were synthesized in bulk via the ring-opening polymerization (130 °C; 72h) in the presence of zirconium (IV) acetylacetonate. The rods with ARP (Zhejiang Huahai Pharmaceutical Co. Ltd, Linhai City, China) (10% w/w) based on D,L-PLGA (50:50) (D,L-PLGA-50:50 rod-ARP) and D,L-PLGA (70:30) (D,L-PLGA-70:30 rod-ARP) were formulated by hot melt extrusion. The hydrolytic degradation of rods was carried out in constant conditions (PBS, pH 7.4; 37 °C; 240 rpm). The following methods and measurements were used: HPLC; NMR; DSC; SEM; water uptake; and weight loss.

Results and Discussion

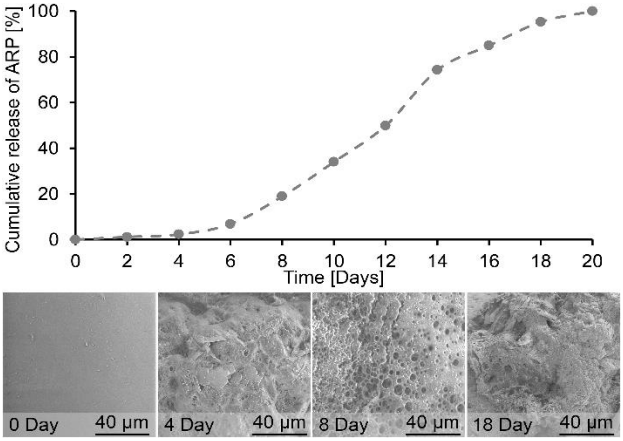

FIG. 1. ARP release profile form D,L-PLGA-50:50 rod-ARP with SEM images of rods during degradation.

ARP was released as a tri-phasic model without a burst effect (FIG. 1 and 2) reflecting the native structure of copolymers and the structural changes during rod degradation (TABLE 1). The higher content of glycolide and the lower glass transition temperature of D,L-PLGA (50:50) resulted in a faster and shorter release compared to rods based on D,L-PLGA (70:30).

TABLE 1. Parameters characterizing the rods during degradation.

D,L-PLGA-50:50 rod-ARP						
Time [Days]	0	2	6	8	14	20
F_{LL} **[mol%]**	48.5	48.9	50.6	51.5	55.1	59.2
F_{GG} **[mol%]**	51.5	51.1	49.4	48.5	44.9	40.8
T_{m1} **[°C]**	*ND*	54.0	52.5	*52.6*	52.5	54.7
ΔH_1 **[J/g]**	*ND*	4.3	4.1	*9.6*	13.5	9.9
T_{m2} **[°C]**	*ND*	*ND*	*ND*	*ND*	*ND*	134.9
ΔH_2 **[J/g]**	*ND*	*ND*	*ND*	*ND*	*ND*	4.7
T_g **[°C]**	38.8	40.2	32.2	40.5	41.9	45.5
WU **[%]**	0.0	4.0	14.2	19.9	58.8	84.4
WL **[%]**	0.0	32.2	63.6	70.7	75.2	72.5
D,L-PLGA-70:30 rod-ARP						
Time [Days]	0	7	14	21	28	49
F_{LL} **[mol%]**	68.5	69.2	70.9	76.0	76.0	90.5
F_{GG} **[mol%]**	31.5	30.8	29.1	24.0	24.0	9.5
T_{m1} **[°C]**	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
ΔH_1 **[J/g]**	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
T_g **[°C]**	51.4	50.6	43.2	30.1	36.0	35.4
WU **[%]**	0.0	2.8	24.0	45.6	60.1	80.1
WL **[%]**	0.0	5.0	16.8	51.4	79.9	97.8

F_{LL} *and* F_{GG}, – molar percentage of lactidyl and glycolidyl units in the copolymer, respectively; $T_{m1,2}$ – melting temperatures; $\Delta H_{1,2}$ – melting enthalpies; T_g – glass transition temperature; *WU* – water uptake; *WL* – weight loss; *ND* – non-detected.

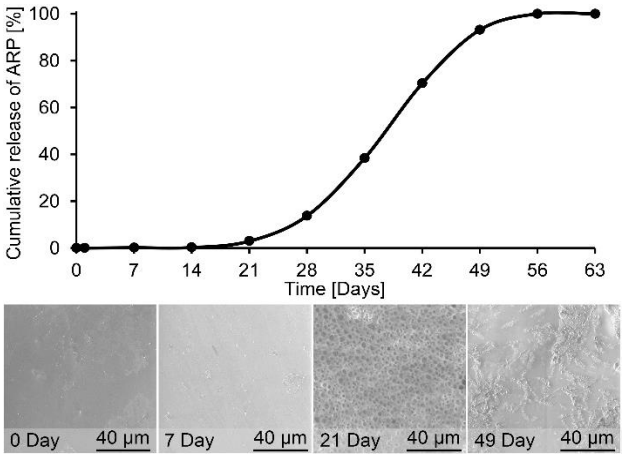

FIG. 2. ARP release profile form D,L-PLGA-70:30 rod-ARP with SEM images of rods during degradation.

Conclusions

The use of rods based on D,L-PLGA (50:50) may allow the elimination of oral supplementation, while the simultaneous use of rods based on D,L-PLGA (70:30) will allow the continuation of therapy.

Acknowledgments

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DEVELOPMENT OF DEGRADABLE TERPOLYMERS RODS FOR PROLONGED RELEASE OF ARIPIPRAZOLE

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Introduction

Aripiprazole (ARP) is an antipsychotic drug substance used in the pharmacological therapy of schizophrenia. Owing to the need for the daily administration of the oral formulations and a lag phase with relatively short release in the case of currently registered ARP long-acting injections, alternative formulations are still being developed [1-3]. This study aimed to formulate a degradable system for the prolonged release of ARP in the form of rods based on degradable terpolymers.

Materials and Methods

Rods with 10% w/w of ARP (Zhejiang Huahai Pharmaceutical Co. Ltd, Linhai City, China) were formulated based on poly(D,L-lactide-*co*-glycolide-*co*-trimethylene carbonate) with a molecular weight (M_n) of 21 kDa (TD,L 21 rod-ARP) and poly(L-lactide-*co*-glycolide-*co*-trimethylene carbonate) with M_n of 59 kDa (TL 59 rod-ARP) were formulated by hot melt extrusion. The hydrolytic degradation was carried out in a PBS buffer (pH 7.4) under constant conditions (37°C; 240 rpm). The following methods and measurements were used: HPLC; NMR; DSC; SEM; water uptake (*WU*); and weight loss (*WL*).

Results and Discussion

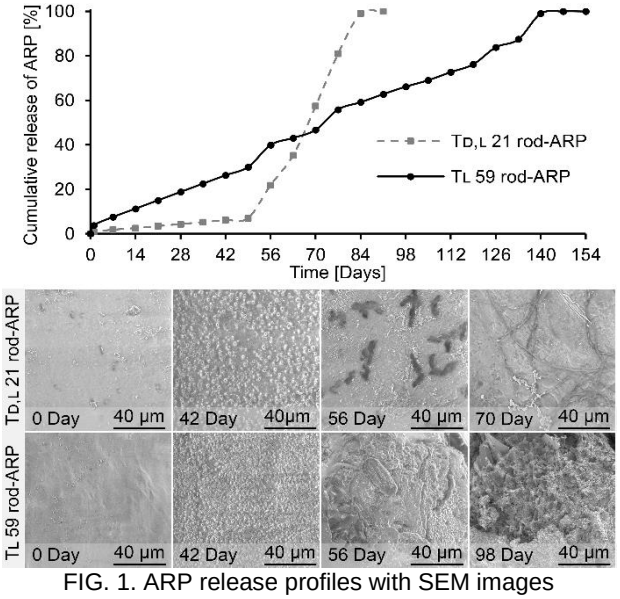

FIG. 1. ARP release profiles with SEM images of the rods during degradation.

ARP was released from TD,L 21 rod-ARP and TL 59 rod-ARP in a tri-phasic model over 91 and 154 days, respectively. The release rate and pattern reflected the structural properties of formulations (FIG. 1). A decrease in the glass transition temperature and an increase in the *WU* and the *WL* were observed. Gradual changes in the terpolymers content were revealed. The most intensive ones were noted for glycolide (TABLE 1).

TABLE 1. Parameters characterizing the rods during degradation.

TD,L 21 rod-ARP						
Time [Days]	0	28	42	56	70	84
F_{LL} [mol%]	63.5	63.1	61.5	62.7	62.1	61.2
F_{GG} [mol%]	15.9	15.5	15.8	12.5	11.5	9.8
F_{TMC} [mol%]	20.6	21.4	22.7	24.8	26.4	29.0
T_{m1} [°C]	*ND*	*ND*	*ND*	*ND*	*ND*	85.2
ΔH_1 [J/g]	*ND*	*ND*	*ND*	*ND*	*ND*	21.6
T_{m2} [°C]	*ND*	*ND*	*ND*	*ND*	*ND*	132.0
ΔH_2 [J/g]	*ND*	*ND*	*ND*	*ND*	*ND*	2.6
T_g [°C]	33.7	33.8	30.9	22.3	26.3	27.2
***WU* [%]**	0.0	3.7	6.3	27.9	61.4	79.1
***WL* [%]**	0.0	5.5	26.8	28.0	32.9	39.2
TL 59 rod-ARP						
Time [Days]	0	28	42	70	98	140
F_{LL} [mol%]	65.4	65.4	64.5	62.9	61.9	74.6
F_{GG} [mol%]	16.3	16.0	15.8	13.2	12.1	9.7
F_{TMC} [mol%]	18.3	18.6	19.7	23.9	26.0	15.7
T_{m1} [°C]	*ND*	*ND*	*ND*	83.4	87.7	92.9
ΔH_1 [J/g]	*ND*	*ND*	*ND*	16.7	39.6	43.2
T_{m2} [°C]	*ND*	*ND*	*ND*	*ND*	139.9	139.1
ΔH_2 [J/g]	*ND*	*ND*	*ND*	*ND*	6.8	6.7
T_g [°C]	41.1	41.2	39.9	38.1	38.5	38.2
***WU* [%]**	0.0	4.9	7.8	34.7	58.5	75.1
***WL* [%]**	0.0	1.5	1.8	26.4	40.7	78.6

F_{LL}, F_{GG}, and F_{TMC} – molar percentage of lactidyl, glycolidyl, and carbonate units in the terpolymer, respectively; $T_{m1,2}$ – melting temperatures; $\Delta H_{1,2}$ – melting enthalpies; T_g – glass transition temperature; *WU* – water uptake; *WL* – weight loss; *ND* – non-detected.

Conclusions

The analysis of rod degradation indicated a stable process that provided the prolonged release of ARP. TD,L 21 rod-ARP could be used as a delayed-release system, while TL 59 rod-ARP could be used as a standard delivery system.

Acknowledgments

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CELL GROWTH KINETICS IN ALGINATE/GELATIN-BASED HYDROGELS

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Introduction

Hydrogels are a popular biomaterial, especially in bioprinting applications. Depending on their composition, they can be mechanically robust, biocompatible, and easily printable matrix for complex biological macro-structures. Alginate and Gelatine based hydrogels are widely used because of their flexibility and ability to tailor their mechanical properties and degradation rates with crosslinking agents [1]. In our study, we wanted to evaluate the growth kinetics and growth patterns of two cell lines (Mice fibroblasts and Human Keratinocytes) in bio-printed hydrogels tailor-made for those cell lines. We have used Alginate/Gelatin-based hydrogels crosslinked with agents such as DAS (dialdehyde stanch) and Calcium Chloride. The hydrogels were based on different culture media depending on the evaluated cell type to ensure optimal growth conditions for the tested cell lines.

Materials and Methods

Two cell lines were used: Mice Fibroblasts (NIH/3T3 - CRL-1658, ATCC) and Human immortalized Keratinocytes (Ker-CT - CRL-4048, ATCC). Cells were cultured and passaged in accordance with the manufacturer's specifications. Hydrogels were based on media specifically used for the correlating cell line; for 3T3 - DMEM/F12K media with 10% FBS (Corning), KBM Gold (Lonza) for KerCT. After mixing freshly made hydrogels (6% gelatin, 2% alginate, 1% DAS – in relation to gelatin) with the cell lines, hydrogels were printed in layers 100-200µm thick and crosslinked with 1% $CaCl_2$. After washing with physiological saline, the manufactured bioprints were incubated in the same conditions as the cell lines used for the bio-printing process. To ensure proper cell growth, the prints were incubated in the correct cell growth media that was replaced every three days. Testing the biocompatibility of the hydrogels during different time points was done with a modified Live/Dead assay. The macro-morphology, growth kinetics, and behaviors of the prints were evaluated using an IX83 inverted microscope with a modified optical path to increase the contrast of the imaging and adapt it for the imaging of hydrogels.

Results and Discussion

Biocompatibility results for both tested biomaterials were sufficient, and after 72 hours, cell viability over 70% was noted. Cell growth behavior for both tested cell lines was similar and depended on the tested hydrogels' degradation rate. Rapid cell growth was achieved three weeks after the start of the experiment. However, we have observed slower growth rates for the KerCT cell line and, in this case, a faster degradation rate for the tailor-made hydrogel. It is understandable since KerCT cells have a lower doubling rate than 3T3 overall. The faster degradation rate of the hydrogel based on KBM media might have been caused by the fact that it is a low-calcium synthetic media. Hydrogel degradation rates might be affected by the presence of cells and the cell growth process, and it was similarly noted by other researchers [2]. We have observed a similar cell 3D structure distribution pattern in the hydrogels for both cell lines. As shown in FIG. 1, the distance between the outer parts of the hydrogel did affect the growth of the cells.

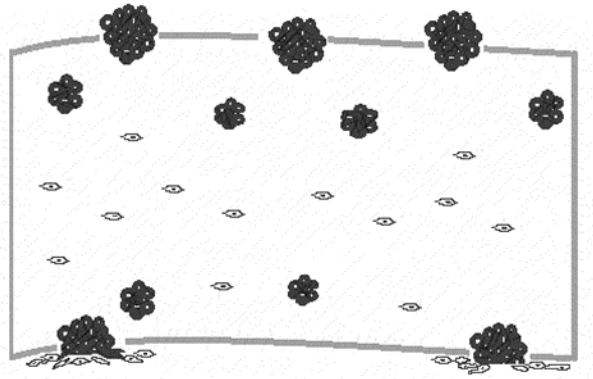

FIG. 1. Cell growth patterns and structure morphology in tested hydrogels. Figure represents a cryosection view representation.

Imaging did prove that the outer surfaces of the hydrogel degrading over time did affect 3D structure formation, such as spheroids. Those were also more prone to detach from the main hydrogel body and migrate toward the bottom of the culture vessel. Spheroid formation in hydrogels based on alginate was also observed by other researchers [3].

Conclusions

Both tailor-made hydrogels gave acceptable biocompatibility that ensured growth of 3T3 and KerCT cell lines. The overall growth behavior between cell lines was similar, and dependent on the degradation rate of the hydrogel. Both spheroids and more complex 3D structures originating from spheroids were observed. The growth kinetics of cells in the printerd hydrogels differ between 3T3 and KerCT cell lines and more favorable cell growth behavior is observed for the cell line with the higher doubling rate.

Acknowledgments

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BIODEGRADATION CHITOSAN FILM MODIFIED WITH CINNAMIC ACID AND ELLAGANIC ACID

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Introduction

The traditionally used plastics, with their remarkable durability and resistance to microbial degradation, result in the accumulation of unnecessary waste in the environment (1). Therefore, alternative degradable polymers are being sought. Chitosan has attracted attention not only for its non-toxicity but, most importantly, for its potential biodegradability. It is also a material that lends itself to modification to improve its properties (2).

Materials and Methods

For the study, pure chitosan and chitosan modified with cinnamic acid and ellagic acid at concentrations of 0.2% and 0.4% were used. The films were prepared at the Department of Biomaterials and Cosmetics Chemistry, Faculty of Chemistry. Fungi of the genus Trichoderma designated T.01, T.05, T.07, T.11, T.14, and T.15 were selected to test their ability to degrade chitosan films. Fungi of the genus *Trichoderma* were obtained from the Department of Biochemistry and Microbiology, Warsaw University of Life Sciences. Microbiological tests included: (1) determination of enzymatic activity of fungi of the genus *Trichoderma* by fluorometric method, (2) determination of the ability of fungi to utilize chitosan films by respirometric method, (3) selection of the most enzymatically active fungi with the highest capacity to decompose chitosan film for the preparation of a microbial preparation, (4) determination of the biodegradation of chitosan film in soil using a *Trichoderma* biopreparation.

Results and Discussion

Enzyme activity (U/h) of *Trichoderma* was determined by the release of fluorogenic substrates from MUF-labelled molecules (chitinase, lipase and glucosidase) and MCA (aminopeptidase). *Trichoderma* produced enzymes with different activities, but the activity of chitinases responsible for the degradation of chitin was the highest.

TABLE 1. Enzyme activity of *Trichoderma*, P-aminopeptidase, L-lipase, Chit-chitinase, α-gluc-α-glucosidase, β-gluc – β- glucosidase.

strains	P U/h	L U/h	Chit U/h	α-gluc U/h	β-gluc U/h
T.01	1,58	36,75	19,91	2,39	1,01
T.05	0,95	79,64	17,27	1,98	5,05
T.07	2,15	45,78	3,03	2,18	14,39
T.11	1,75	46,19	45,47	11,78	17,38
T.14	1,45	62,21	31,27	9,91	1,09
T.15	1,47	77,88	69,77	2,7	2,08

Biological oxygen consumption of *Trichoderma* in the presence of chitosan films was observed only in T.07 and T.14.

TABLE 2. Biodegradation [mgO_2/l] of chitosan films by *Trichoderma* CTS-chitosan, CTS + AC - chitosan modified with cinnamic acid, CTS+AE - chitosan modified with ellaganic acid.

Chitosan films	Strains					
	T.01	**T.05**	**T.07**	**T.11**	**T.14**	**T.15**
CTS	0	0	126	0	70	39
CTS + 0,2%AC	0	0	98	0	25	59
CTS + 0,4%AC	0	0	8	0	60	0
CTS + 0,2%AE	0	0	58	0	90	70
CTS + 0,4%AE	0	0	37	0	0	0

Biodegradation of chitosan film modified with cinnamic acid and ellaganic acid was only effective when *Trichoderma* was introduced into the soil. Soil microorganisms degraded the films tested, but with less efficiency. The study also showed that even the introduction of single *Trichoderma* strains into the soil was as effective as the T.07 and T.14 consortium.

TABLE 3. Biodegradation of chitosan film modified with cinnamic acid and ellaganic acid after bioaugmentation of *Trichoderma* [mgO_2/kg of soil]. CTS-chitosan, CTS + AC - chitosan modified with cinnamic acid, CTS+AE - chitosan modified with ellaganic acid.

Chitosan films	Variants			
	native microorganisms	T.07	T.14	MIX
CTS	2089	3184	2675	2929
CTS + 0,2AC	1758	2140	1910	2165
CTS + 0,4AC	484	611	662	917
CTS + 0,2AE	1299	1426	1401	1885
CTS + 0,4AE	637	1350	1121	1401

Conclusions

Studies have shown that chitosan film modified by cynnamic and ellaganic acid is better degraded after application by fungi of the genus *Trichoderma*. Indigenous microorganisms in the compost degraded the chitosan films poorly.

Acknowledgments

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THE CHARACTERIZATION OF COLLAGEN/BETA GLUCAN HYDROGEN MODIFIED BY TANNIC ACID

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Introduction

Around 40% of fish weight is considered as by-products. The utilization of these by-products is still limited, however they can by considered as good sources of many valuable resources as proteins, fat, minerals ect. (1). The fish skin may be considered as collagen source. It was found that collagen isolated from fishes induced keratinocyte differentiation, which is required for the formation of integrated epidermis (2). To change the collagen properties, collagen may be mixed with different polymers. Based on our previous study, collagen was mixed with beta glucan. And to improve mechanical properties and stability of hydrogels, tannic acid was used as crosslinking agent. We tested the hypothesis that the addition of this antimicrobial agent should act as a crosslinking agent in this mixture.

Materials and Methods

Collagen (Coll) was isolated from the skin of *Hypophthalmichthys nobilis*. The detailed procedure was descripted in our previous work (3). The collagen (1%) and β-glucan (BG) (5%) solutions were mixed in a ratio of 90:10 (w/w). The collagen/β-glucan mixture was modified by adding 2%, 5%, or 10% tannic acid (TA) solution (2%).The resulting mixture was dialyzed against distilled water for 7 days. The interaction between the functional groups of polymers and tannic acid was assessed using ATR-IR. Two key mechanical parameters were evaluated: the maximum compressive strength (σmax) and the compressive modulus (Emod) using Zwick&Roel testing machine. To analyze the release of tannic acid from the hydrogel matrices, the Folin-Ciocalteu method was employed (4). The evaluation of antioxidant properties was conducted using the DPPH reagent.

Results and Discussion

The Coll/BG spectrum showed groups such as Amide A (3292 cm^{-1}), Amide B (2928 cm^{-1}), Amide I (1634 cm^{-1}), Amide II (1521 cm^{-1}), Amide III (1222 cm^{-1}) characteristic for collagen samples. The maximum peak at 1007 cm^{-1} corresponded to glucopyranose moiety, and at 896 cm^{-1}, a characteristic of β-linked glycosidic bonds constituted characteristic adsorption peaks for β-glucan. We did not observe any significant shifts in the FTIR spectra. However, based on the literature we assume that between the hydrogel components, hydrogen bonds and van der Waals interactions are present.

The mechanical properties of hydrogels are presented on FIG. 1. In this study, the concentration of the crosslinking agent did not affect the mechanical properties of the Coll/BG hydrogels. However, it should be noted that the hydrogels made solely with Coll/BG were not solid and therefore were not suitable for direct comparison. Based on this observation, it can be inferred that the crosslinking process enhanced the mechanical properties of all the crosslinked hydrogels prepared in this study.

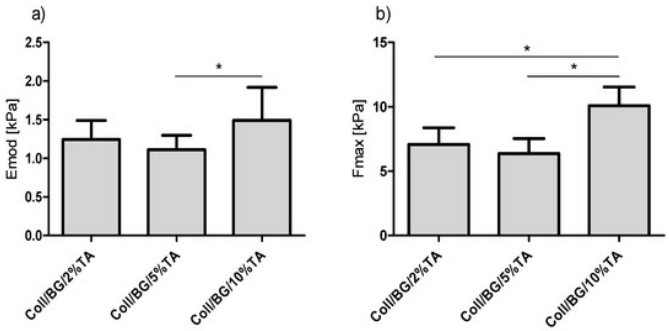

FIG. 1. The mechanical parameters of hydrogels with different tannic acid concentrations: (a) comprehensive modulus; (b) maximum tension; * $p < 0.05$ between groups.

The release of tannic acid occurred gradually over time without any sudden bursts or spikes in the release profile. This characteristic is advantageous as it allows for the development of controlled delivery systems. Controlled release systems provide a more predictable and sustained release of tannic acid, enabling precise dosage and therapeutic applications.

TABLE 1. The antioxidant properties of the hydrogels during different incubation times.

Specimen	After 1.5 h of contact [%]	After 18 h of contact [%]	After 24 h of contact [%]
Coll/BG + 2%TA	0.23 ± 0.01	0.84 ± 0.04	20.16 ± 0.08
Coll/BG + 5%TA	0.91 ± 0.03	62.08 ± 0.12	58.30 ± 0.17
Coll/BG + 10%TA	7.91 ± 0.02	74.83 ± 0.24	81.06 ± 0.28

TABLE 1 presents the antioxidant properties of the prepared materials, expressed as the percentage of free radical scavenging. The observed antioxidant activity was influenced by the concentration of tannic acid and the duration of sample incubation. Tannic acid possesses the ability to scavenge free radicals due to the presence of phenolic hydroxyl groups. These hydroxyl groups have the capacity to reduce DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radicals, thereby exerting antioxidant activity.

Conclusions

Stable collagen/β-glucan hydrogels may be obtained by using tannic acid as crosslinking agent. This natural crosslinking agent improve the mechanical properties of hydrogels. By prolonged time of tannic acid release, the biological properties of hydrogels may be improved, as well as tannic acid addition improve antioxidant activity od prepared materials.

Acknowledgments

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BIOPOLYMERIC SCAFFOLD - MEDIATED BONE REPAIR

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Introduction

Tissue engineering, evolved from biomaterials development, involves combining scaffolds, cells, and biologically active molecules to create functional tissues. Orthopedic medicine faces challenges with bone defects from trauma, disease, or surgery. Scaffold-based tissue engineering offers promise for bone repair, aiding cell attachment, growth, and guiding new bone tissue. Repairing large bone defects remains a challenge, as single-component biopolymers fall short. Multi component biopolymeric scaffolds hold potential, but compatibility issues can weaken mechanical properties. Various fabrication techniques and optimizations with biomaterials and crosslinking have been explored, alongside advancements in fabrication methods. Bio-fillers in solvent casting are also studied for dispersion and mechanical properties.

Materials and Methods

Scaffold fabrication techniques included solvent casting, which used solvent evaporation to create porous constructs, and electrospinning, which produced nanofibers with high surface area and porosity. A series of methods were employed to fabricate scaffolds for tissue engineering. Poly (vinyl alcohol) (PVA) a water-soluble, biocompatible polymer with high hydrophilicity making it suitable for tissue engineering, particularly in joint cartilage replacement and Poly Lactic Acid (PLA) was chosen for its hydrolytic degradation properties, were used as base polymers, with the incorporation of Tricalcium Phosphate (TCP), Bioglass (BG), to enhance scaffold properties. The materials are purchased from sigma Aldrich. Solvent casting, sonication, and other techniques were utilized in various combinations to achieve the desired scaffold structures. Results varied across the trials, with factors such as uniformity, dispersion, and thickness influencing the outcomes. Modifications in the scaffold preparation process, including the use of plasticizers and changes in solvents, were explored to optimize the scaffold properties. The experiments highlighted the importance of material compatibility and processing methods in scaffold development for tissue regeneration applications.

Results and Discussion

Solvent cast PLA films are concerned, the absence of mechanical shear and the slow solvent evaporation cause the polymer to settle on the bottom of the petri dish, while the TCP remains on the top layer, leading eventually to a coating layer of about few micrometres on the film surface. The presence of tubular pores can be seen from the FIG. 1 (SEM under various magnifications). The mechanical properties of the PLA/TCP composite material provide details about its performance under tensile loads. In FIG. 2 the maximum load of 3.995 kgf is the material's breaking point, while the 60.207 mm extension at this load showcases its ductility. Tensile stress at 61.468 kgf/cm² gauges its resistance to stretching, and tensile strain at 3.010 kgf /cm² reveals how much it deforms relative to its original length. Young's modulus, at 58.60880 kgf/cm², signifies its stiffness. These properties collectively determine the material's suitability for applications requiring tensile strength and deformation characteristics.

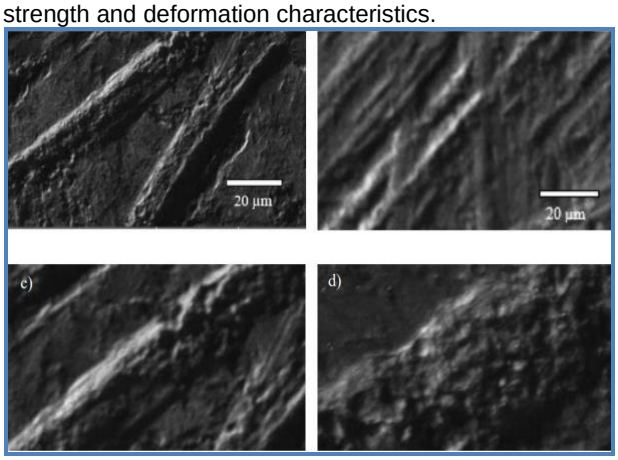

FIG. 1. Cross sectional SEM image of PLA/TCP showing tubular pore at 20 µm.

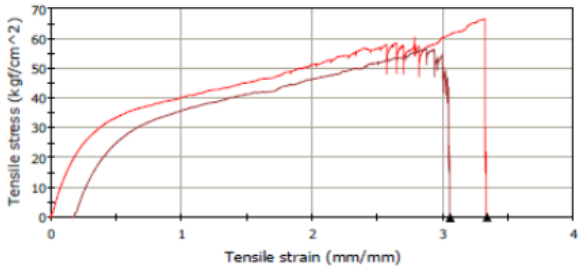

FIG. 2. Stress Strain graph for PLA/TCP Film.

Conclusions

Overall, the study shows that PVA/PLA scaffold preparation was successful. The polymer matrix composition was further optimized by including different filler systems. Various literature evaluations were conducted to investigate the impact of various manufacturing techniques on scaffold preparation. The usage of polylactic acid and polyvinyl alcohol with various bio filler systems such as tricalcium phosphate and bio glass resulted in the successful incorporation of solvent casting. In the future, we intend to release gasotransmitters and incorporate growth factors, as well as to enhance the scaffold composition as a smart scaffold for bone tissue applications.

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PRELIMINARY EVALUATION OF CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY OF LINEAR POLYAMIDOAMINES SYNTHESISED WITH USE OF EXOGENOUS POLYAMINES

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Introduction

The observed current development of pathogens and the increase in bacterial resistance is concerning. Modern surgery also struggles with a significant problem of bacterial infections [1]. Escalating issues necessitate the development of new biomaterials that protect against antimicrobial activity. Polyamines, primarily endogenous ones with known antibacterial and antiviral properties, are highly promising for use in creating biomaterials with antibacterial properties [2]. Furthermore, due to their possession of amino groups, polyamines and their derivatives allow for further modifications, expanding the range of potential biomedical applications. This paper presents a preliminary biological assessment (cytotoxicity studies and activity against selected bacteria) of the polyamidoamines based on putrescine, spermidine, norspermidine, and spermine obtained by interfacial polycondensation with sebacic acid dichloride. The obtained polyamidoamines present interesting properties. Undoubtedly, these materials can have a wide range of applications in medicine and cosmetics.

Materials and Methods

A series of linear polyamidoamines based on putrescine (PA), norspermidine (PAA1), spermidine (PAA2), and spermine (PAA3) with protected secondary amino groups were obtained using sebacic acid dichloride by an interfacial polycondensation process. The obtained polyamidoamines were characterized with NMR, FTIR, and DSC techniques, and the water contact angle was measured. The cytotoxicity study has been conducted according to the ISO 10993-5 standard. The human fibroblasts WI-38 (CCL-75) were obtained from ATCC and the human keratinocytes (HaCaT) were purchased from Cell Line Service (CLS). The evaluation of antibacterial and antifungal activity was conducted according to the recommendations of the Clinical and Laboratory Standards Institute. The following strains were selected for the study: Gram-positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis. Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa. Fungi: Aspergillus brasiliensis. Yeast: Candida albicans.

Results and Discussion

Polyamines with protected secondary amino groups enabled the synthesis of linear polyamidoamines with an average molecular weight above 5000 g/mol. The NMR and FTIR measurements confirmed the presence of amide and amino groups in the main chain of obtained polymers. The conducted studies approved only moderate cytotoxicity of the synthesized polyamidoamines. Polyamines with protected secondary amino groups and active primary enabled the synthesis of linear polyamidoamines. The deprotection of these groups was done after the purification of the final polymer. The antibacterial activity of the obtained polyamides was evident. This was particularly noticeable against E. coli, where practically all polymers have shown strong inhibitory effects on the growth of this strain (FIG. 1). The polyamidoamine PAA3 contain derivatives spermine in the polymeric chain and exhibits the broadest activity. Surprisingly, the polyamide PA obtained with putrescine also shows significant activity against Staphylococcus aureus (FIG. 2). Furthermore, the polyamides obtained with spermine and putrescine also exhibit antifungal properties. The particularly high antibacterial activity of the polyamidoamine with spermine derivative can be attributed to the presence of a large number of amino groups in the chain of this polymer.

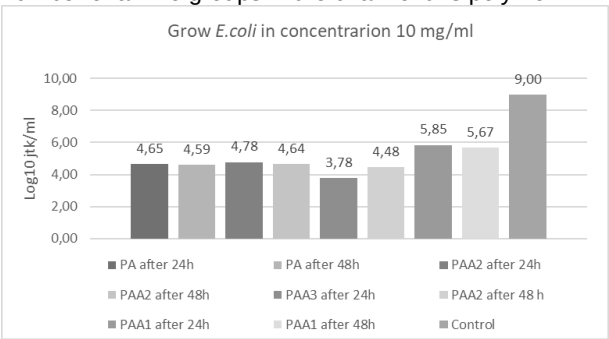

FIG. 1. Comparison of the growth of *E. coli* strains against polyamidoamines within 24 and 48 hours of incubation.

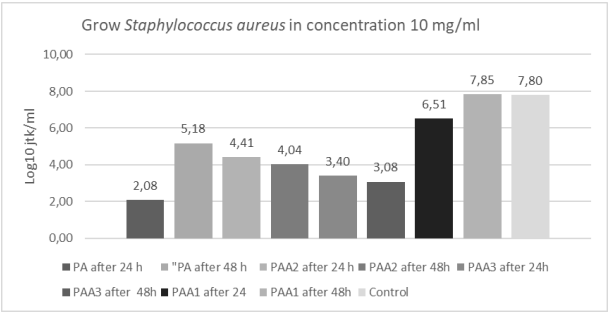

FIG. 2. Comparison of the growth of *Staphyloccus aureus* strains against polyamidoamines within 24 and 48 hours of incubation.

Conclusions

Based on the conducted studies, it can be concluded that the obtained polyamidoamines have great potential as new bioresorbable material. The research results encourage further experiments, modifications, and exploration to expand their applicability as a new class of antibacterial biomaterials for use in tissue engineering, and as a drug carrier, or in wound dressing.

Acknowledgments

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INFLUENCE OF SUBSTRATE PREPARATION ON ADHESION STRENGTH, MORPHOLOGY AND SURFACE PROPERTIES OF SODIUM ALGINATE COATINGS

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Introduction

Stainless steels are commonly used materials in biomedical engineering. They are relatively inexpensive, have low tissue reactivity, and electrochemical corrosion resistance and mechanical strength sufficient for short-term orthopaedic implants. Currently, peri-implant infections are a serious problem, also in the case of short-term implants. One of the ways to prevent them is to coat metallic implants with natural coatings. One of the prospective natural biopolymers is sodium alginate (SA). This water-soluble polysaccharide is extracted from brown algae and is biodegradable, biocompatible and non-toxic [1]. A suitable method for the development of this type of coating is electrophoretic deposition (EPD). The process is carried out at room temperature, so there is no risk of damage to the polymer, and dispersing phases that are safe for the natural environment are used [2]. The main objective of this work was the EPD of SA coatings on stainless steel substrates after various surface treatment (mechanical and chemical), and to determine the effect of surface treatment on the morphology and adhesion strength of the coatings.

Materials and Methods

Austenitic AISI 316L stainless steel with six different surface preparation routes was used as a substrate: 1 – as-received, 2,3 – after sanding with 600 and 1200 grit paper, respectively, 4 – after etching as described in [3], 5,6 – after silanization with and without heat treatment (HT), respectively, as described in [4]. The suspension for EPD was prepared by dissolving SA in distilled water. The solution was then ultrasonically dispersed for 10 min. and ethanol was slowly added. The SA concentration was 4 g/l, while water and ethanol were used in a 60/40 ratio. EPD was carried out in a standard two-electrode system. The coatings were deposited at a constant voltage in the range of 1-8 V for 240 s. During EPD, the current density was measured with the EPD time. Morphology investigations of the materials were performed using LM and SEM. The surface topography and roughness of the materials were examined using optical profilometry. The adhesion of the coatings to the substrates was investigated using the cross-cut tape test method in accordance with the ASTM D3359 standard. Contact angle (CA) and surface free energy (SFE) were measured for both substrates and coatings.

Results and Discussion

All coatings had high uniformity after EPD at 4 V for 4 min., regardless of the substrate used. The morphology of all coatings was homogeneous. The SA evenly coated the surfaces of all substrates, without cracks and voids.
The lowest adhesion (class 0B) showed the coatings deposited on the substrates in the delivery state, sanded on 600 grit paper and after silanization with HT. The adhesion class of the coatings on silanized substrates without HT was 3B (small flakes of the coating are detached along edges and at intersections of cuts). The coatings showed high adhesion (class 4B, only small flakes of the coating are detached at intersections) to the substrates sanded with 1200 grit paper and etched ones. To explain the effect of substrate preparation on the adhesion strength of coatings, roughness, surface topography and wettability were investigated. In general, surface treatments developed rougher surfaces (except for silanization with HT) compared to as-received steel (R_a=0.29±0.03 µm and R_q=0.38±0.04 µm). The etched surfaces exhibited R_a=0.42±0.16 µm and R_q=0.52±0.18 µm. Substrates sanded on 1200 grit paper and silanized (without HT), had the highest roughness (R_a=0.59±0.24 µm and 0.51±0.03 µm as well as R_q=0.77±0.33 µm and 0.64±0.04 µm, respectively). The coatings deposited on rough surfaces (ground, etched and silanized without HT) had a relatively high adhesion. The smooth surface of the substrate silanized with HT (R_a=0.12±0.01 µm, R_q=0.16±0.01 µm) did not allow a strong bond to form with the coating. In addition, the etched substrates had slightly lower R_a, but a much higher R_t (9.5±2.2 µm) than the ground and silanized without HT ones (R_t=6.4±1.2 µm and 6.6±0.4 µm), which indicates the presence of larger depressions on the surface. They allow the coatings to be mechanically interlocked on the steel surface. These results indicated that roughness has a much higher impact on the adhesion strength of coatings than the chemical composition of the substrate surface.
All substrates and coatings exhibited a slight and moderate hydrophilicity, respectively. CA for water for all substrates (62.8°-82.6° depending on the substrate) was significantly higher compared to that with CH_2I_2 (36.5°-51.8°). In the case of coatings, the differences were not as large, and the CA in most cases was slightly higher for CH_2I_2 than for water (44.3°-52.5° and 29.5°-49.7°, respectively). For all substrates, the SFE values were quite high for the disperse component (above 30 mN/m) and low for the polar component (below 14 mN/m). The coatings, regardless of the substrate, were characterized by relatively high SFE values (around 60 mN/m), with a similar share of dispersive and polar components. The polar component is responsible, among others, for the bond strength.
Considering the relatively poor surface development of the substrates, the coatings exhibiting medium hydrophilicity are able to wet the surface well and contribute to their high adhesion.

Summary

The surface preparation of the steel substrates had a significant impact on the adhesion strength of the coatings. Coatings deposited on rough substrates had the highest adhesion strength. Thus, mechanical interlocking rather than chemical bonding may be mainly responsible for the adhesion strength of coatings. The surface treatment of the substrate did not affect the homogeneity of the coatings. All investigated surfaces, including substrates and coatings, showed a contact angle below 90°, with the highest being for water. The wettability of the substrates did not have a significant influence on the coating adhesion.

Acknowledgments

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CALCIUM PHOSPHATE COATINGS ON TITANIUM PRODUCED USING AN ULTRASONIC MICRO-ARC OXIDATION PROCESS

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Introduction

An aging society, a sedentary lifestyle, road accidents, falls from heights, and sports injuries are some of the many causes of musculoskeletal disorders [1,2]. Bone, as a rigid organ of the skeletal system, can perform dynamic biological remodeling involving osteoclasts and osteoblasts. As a result, bone self-regeneration is possible, but if the damage is too large (> 2 cm) surgical intervention is required [3,4].

Titanium is a material widely used in bone tissue engineering because of its good biocompatibility, chemical stability in the environment of body fluids, and high corrosion resistance. However, there are still problems with no bioactivity and reasonable mechanical properties. Porous coatings with suitable chemical and phase compositions are generated on their surface to improve their characteristics. Their properly designed has a beneficial influence on bone cell adhesion, proliferation, and differentiation [5].

One of the techniques that enable the modification of titanium implants is micro-arc oxidation (MAO). This electrochemical method enables the deposition of coatings with different microstructures and chemical compositions and does not require specialized and expensive equipment [5]. Modifying the process parameters and the composition of the electrolyte allows for adjusting the functionality of the coatings, and the use of ultrasound (UMAO) also utilizes to increase the energy impact during MAO [6]. The research aimed to select a technology that would enable obtaining coatings that improve titanium materials' properties in regard to biomedical applications.

Materials and Methods

The research used commercially pure titanium (CP-T) samples for micro-arc oxidation and ultrasound micro-arc oxidation processes. The MAO and UMAO process was performed using DC power supplies (MR100020, M&K Precision Corp.) under process parameters: 400 V, 450 s or 600 s, 120 mA/cm^2, and 60 mA/cm^2 in an electrolytic solution containing 14 g/L calcium acetate hydrate and 3 g/L β-glycerophosphate disodium salt pentahydrate – based on a literature review [5]. Two ultrasound (US) operating modes (sinusoidal and unipolar square wave) were employed. Details of MAO and UMAO coatings formed with different parameters are listed in TABLE 1.

To obtain the characteristics of coatings, the following research was performed: 1) the morphology by SEM microscopy (JSM-7800 F, JEOL Ltd., Japan); 2) the elemental composition (EDS, Edax Inc., USA); 3) the surface wettability by optical tensiometer (optical tensiometer, Attention Theta Life); 4) nanomechanical properties (Alemnis AG, Thun Switzerland); 5) coating adhesion by scratch test (NanoTest Vantage, Micro Materials Ltd.); 6) the cytocompatibility – assessment of hFOB 1.19 cell line viability (ATTC-CRL-11372) by MTT and LDH assay; 7) corrosion behavior by potentiostat/galvanostat (Atlas 0531, Atlas Sollich, Poland).

TABLE 1. The labels of MAO and UMAO coatings formed at different conditions.

Label	Voltage [V]	Current [mA]	Time [s]	Ultrasounds
CP-Ti	-	-	-	–
136_450	400	136	450	–
136_450_sin				sinusoidal
136_450_sq				unipolar square
136_600			600	–
136_600_sin				sinusoidal
136_600_sq				unipolar square
68_450		68	450	–
68_450_sin				sinusoidal
68_450_sq				unipolar square
68_600			600	–
68_600_sin				sinusoidal
68_600_sq				unipolar square

Results and Discussion

In all cases, porous Ca- and P-containing titania-based coatings were successfully formed on the CP-Ti samples. Coated samples were characterized by a more preferable wettability and better corrosion resistance than uncoated materials regarding biomedical applications. All coatings exhibited good adhesion to the substrate, whereas their morphological, physical, and mechanical properties were highly dependent on the process parameters. The UMAO coatings were characterized by an extensive net structure and a large number of volcano-like pores. In addition, the use of ultrasound increased the porosity and pore size of the coatings. Generally, ultrasound enables augmented incorporation of calcium into the coating, while the amount of phosphorus for all samples remained at a similar level. All specimens were hydrophilic inasmuch as the contact angle of MAO coatings was 30-40°, and UMAO coatings ranged from 9° to 45°. Furthermore, cytocompatibility studies confirmed that except for sample 68_600, the other coatings have no adverse effect on human osteoblasts. The most optimal properties for applications in hard tissue engineering are characterized by the sample 68_450_sin.

Conclusions

The results show that the surface treatment of titanium is critical for biomedical applications. The characteristics of the coatings are related to the parameters used during the processes but also to the characteristics of the ultrasound. The combination of MAO with US is a promising method to improve the properties of commercially pure titanium materials dedicated to biomedical applications, in particular for modifying titanium implants for (i) partial joint resurfacing of the knee, (ii) spinal interbody fusion, or (iii) craniofacial reconstruction.

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ON THE ROUTE OF ADHESION IMPROVEMENT FOR POLYPYRROLE COATING

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Introduction

Medical steel is utilized in many fields including medical implants like electrodes used for tissue stimulation or stainless steel plates for bone fixation [1]. To achieve sustainable applicability of such devices the proper corrosion protection ability shall be assure especially under various biological conditions. Such prevention can be achieved by covering of the implant body with additional coating of polymer nature. One of the most effective coating in this respect is composed of polypyrrole as matrix [2] commonly enriched with additives like ZnO [3], elastomer based systems [4] or natural orgin additives like saccharinate [5]. In all application the adhesion of the coating is crucial to assure proper protection as well as functionality while being an interlayer for effective electron transport in neural applications [6]. In the work we propose the methodology to improve pristine adhesion of the pPy coating.

Materials and Methods

Materials were deposited on the surface of the medical 316 L steel from the solution of pyrrole (0.2 M) and optimally bioorganic modificator namely tannic acid (0.001 M). Electropolymerisation and electrochemical characterisation of the polymer films were performed using an AUTOLAB PGSTAT 12 potentiostat. Measurement data were recorded using Autolab GPES 4.9 software. The experiments were carried out in an electrochemical cell equipped with three electrodes set namely working electrode (steel 316 L), reference electrode (Ag/AgCl) and counter-electrode (Pt spiral). All potential values given are relative to electrode R. The samples were deposited by CV protocole which serves as a potentiodynamic mode. In the experiment adhesion and strength of the bonding for the coatings were tested with 2 approach. The first one was a modified ASTM tape adhesion test with the use of low-medium adhesion Scotch 3 M Blue masking tape for delicate surfaces. The images before and after tape removal were recorded and served for comparison. The sclerometric test revealed the scratch resistance of the coating by using the Micro Combi Tester - MCT3 from Anton-Paar (Corcelles-Cormondrèche, Switzerland) by scratch-testing. The tests were carried out in accordance with ISO 19252, ISO 20502, ASTM C1624 and ASTM D7027 using a Rockwell diamond indenter with a diameter of 100 µm.

Results and Discussion

The CV curves recorded in the sodium salicylate (NaSal) as electrolyte for pure monomer solution (FIG. 1) show a growth of the current density in the first scan in the region of potential corresponding to monomer oxidation (appr. 0.8 V). In the subsequent scan the charge density is lower as the process of polymerization starts after induction time where both oligomer density grows in the proximity of the electrode and some introductory nucleation acts take place. Only after than the current start to grow leading to extending of the chain length with their deposition on the steel surface. Presence of the bioorganic modifier (itaconic acid) do not change the path markedly. Deposition of the black layer visually confirms pPy formation. Still close inspection of value of the charge passed through the electrode revealed slight decrease in polymerisation efficiency after adding modifier.

The tape test showed that for used condition both samples prove to be adherent as only tiny amount of the material was peeled with the adhesion tape. Still the amount for modified sample is lower in comparison to the unmodified one.

In the field of sclerometric test two critical loads were determined on the samples being load at which the first plastic deformation of the polymer layer occurred (Lc1), load at which the first damage to the polymer layer occurred, e.g. deformed micro-cracks of the conformal type appeared (Lc2) and load at which complete rupture of the polymer layer occurred (Lc3). In the procedure the loading in a range of 0.03: 10.0 N was used with loading rate of 20 N/min, while 2 scratches were done (FIG. 2). The value of critical loading Lc1 for non-modified sample was 0.32 N, while for modified one – 0.63, Lc2 for non-modified sample was 2.85 N, while for modified one – 3.89 N and Lc3 for non-modified sample was 4.06 N, while for modified one – 5.58 N.

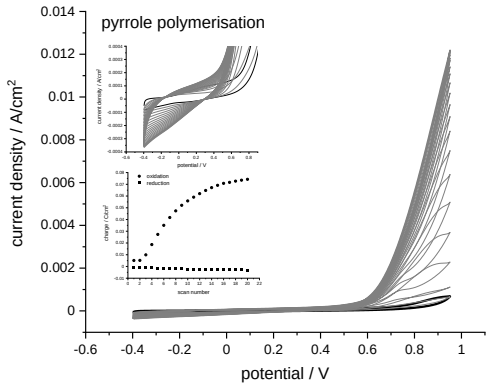

FIG. 1. CV curves recorded during pyrrole polymerization in the presence of itaconic acid in the sodium salicylate solution (condition: 20 cycles, 0.05 V/s, S_w = 0.64 cm^2).

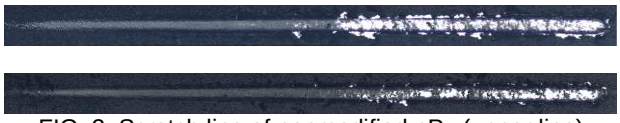

FIG. 2. Scratch line of nonmodified pPy (upper line) and pPy modified with IA (lower line).

Conclusions

The presence of bioorganic modifier at the synthesis stage produced the material of improved adhesion as evidenced by both the sclerometric and adhesive tape tests. Improved adhesion is highly beneficial from application point of view.

Acknowledgments

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POTENTIAL OF GLUCOSAMINE SULFATE AND CHONDROITIN SULFATE IN THE POLYLACTIDE-POLYCAPROLACTONE SCAFFOLDS FOR REGENERATION OF OSTEOCHONDRAL DEFECT

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Introduction

The biomimetic approach based on imitating the structure of natural tissues and processes occurring in them in the design of the composition and parameters of implants seems to be the most biocompatible and promising in tissue regeneration. It allows to naturally reproduce the chemical composition of tissues, their structure and microstructural architecture and their properties, such as mechanical and biological. An ideal scaffold in tissue regeneration should temporarily provision the repair processes of the treated tissue by inducing appropriate biological processes and ensuring a mechanical supporting for the overgrowing tissue. This can by realized by modification of degradable polymers (polylactide and polycaprolactone) by substances occurring naturally in cartilage and subchondral layer, which are responsible for its regenerative processes. Such substances include chondroitin sulfate and glucosamine sulfate. Chondroitin sulfate has an ability to adsorption of growth factors and proteins [1]. Moreover, it is characterized by anti-inflammatory and antioxidant effects, and its presence in cartilage tissue is a signal to start the process of chondrogenesis. Glucosamine is one of the most important substrates in the process of biosynthesis of glycosaminoglycans and proteoglycans present in the extracellular matrix of cartilage tissue. Glucosamine have chondroprotective properties and reduce the secretion of proinflammatory cytokines [2]. The interest in these materials in the modification of substrates is reflected in numerous research works [3].
An innovative approach in the presented research concerns the development of a multiphase scaffolds reproducing natural gradients occurring in cartilage tissue, taking into account the bottom of the subchondral layer. This is due to the fact that typical techniques used to stimulate cartilage regeneration is drilling, aimed at creating contact with the bone area with significant regenerative potential (presence of metabolically active cells, vascularization and innervation). Therefore, in addition to glucosamine sulfate and chondroitin sulfate, hydroxyapatite (HAp) particles were also used as a modifier.

Materials and Methods

The following materials purchased from Sigma-Aldrich were used: polylactide (PLA) 3251D, polycaprolactone (PCL) 80,000Mn, glucosamine sulfate (GS), chondroitin sulfate (CS), hydroxyapatite (HAp) ACROS.
In the first step, a 10% (w/v) solution of PLA and PCL (1:1) in 1,4-dioxane was obtained. In the next step, solutions were cast into a mold and frozen for 30 min at -20°C, containing respectively: 5 wt.% of GS (sample 1), 1 wt.% of GS (sample 2), 1 wt.% of GS and 0.15 wt.% of CS (sample 3). Then, a solution containing 10 wt.% of HAp was poured onto the frozen materials to create the second layer. The mold with solutions was frozen again at -80°C for 24 hours. Then the materials were subjected to a freeze-drying process (LABCONCO, 47°C, 48h, 0,08mBar). Sample 1 and sample 2 were also obtained in a single-layer arrangement. PLA/PCL without modifiers was used as a reference.
Microstructure of samples were observed using Scanning Electron Microscopy SEM (FEI Nova NanoSEM 200). Mechanical studies were performed using universal testing machine (Zwick 1435) in the compression test. Samples were incubated in phosphate buffered saline (PBS) in 37°C. The study periods were set at 3 and 6 weeks. All studies were triplicate for statistical analysis.

Results and Discussion

Studies have shown that the addition of glucosamine sulfate significantly affects the porosity of scaffolds (reducing pore size and increasing porosity) and mechanical parameters such as compressive strength and compression modulus (clearly increasing the compression modulus). This can probably be associated with the nucleation of more water crystallites during freezing in the presence of GS, while at the same time their smaller growth. The effect of CS was not as pronounced in terms of these parameters, but the addition of HAp increased the compressive strength and compression modulus of the samples to the greatest extent. Importantly, the simultaneous use of chondroitin sulfate and hydroxyapatite significantly increased the bioactive potential of these scaffold (much more apatite secretions in incubation in a simulated biological environment was observed). This effect was not visible for scaffold with HAp but without CS addition.
Significant discrepancies in the literature concern the amount of chondroitin sulfate used in biomaterials. It ranges from 0.01 wt.% to 10 wt.% [3]. In the presented study, a CS share of 0.15 wt.% was used, which proved to be sufficient for the induction of nucleation processes and the growth of apatite crystals. Taking into account the assumption that the developed scaffold is to reach the bottom of the subchondral layer (osteochondral defects) due to ensuring access to metabolically active cells, the analysis of bioactive potential plays an important role. However, in subsequent studies, it is necessary to evaluate the developed scaffolds in cell cultures.

Conclusions

The applied modifiers had a positive effect on the mechanical parameters and porosity of the scaffolds. The greatest effect on porosity was observed in the case of GS, while on strength and compression modulus in the case of HAp.
The greatest bioactive potential was demonstrated by a sample containing simultaneously HAP and CS.
The use of all three additives simultaneously (GS, CS and HAP) allowed to achieve the most beneficial properties.

Acknowledgments

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COLLAGEN-BASED SCAFFOLDS MODIFIED BY PHENOLIC ACIDS – BIOLOGICAL STUDIES

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Introduction

Advanced scientific research has led to the dynamic development of biomaterials and the creation of many structures applicable in a wide range of fields, such as dentistry, maxillofacial surgery, ophthalmology, and regenerative medicine, orthopedics, bioengineering, cardiology, and surgery plastic [1]. The aim of the experiment was to study of the biological properties of scaffolds such as hemo- and cytocompatibility, and antimicrobial activity.

Materials and Methods

Collagen (Coll), ferulic acid (FA), gallic acid (GA), and caffeic acid (CA) were dissolved separately in 0.1M acetic acid at 1% concentration. The phenolic acid solution was added to the collagen solution in the 10 w/w% ratios. Then they were frozen by using the lyophilizator (ALPHA 1–2 LDplus, CHRIST,−20°C, 100 Pa, 48 h). The experiments of hemo- and cyto-compatibility of scaffolds were conducted on human red blood cells (RBCs) and human osteoblasts cell lines (hFOB 1.19, ATCC RRID: CVCL 3708). RBCs treated with 2% Triton were used as a positive control (i.e., 100% hemolysis) and RBCs incubated without films as a negative control. According to the literature absorbance values which did not exceed the value of 1 were assumed negative for hemolysis [2]. For proliferation study, cells were seeded at a density of $35x10^3$ cells on a 24-well plate and cultured for 24h at standard conditions. Full protocol was published by us and can be found in the literature [3]. The inhibition of bacterial growth test was carried out according to the McFarland standards. The experiments were performed using two different strains of bacteria: Staphylococcus aureus ATCC 29213and Escherichia coli ATCC 25922. Their initial concentration was set at 1.5×10^8 CFU mL^{-1} (0.5 iMS) [3].

Results and Discussion

All the modified collagen-based scaffolds may be considered biocompatible as no significant negative effect on the human erythrocytes and osteoblastic cells was observed. Human osteoblast cell proliferation was also monitored for seven days (FIG. 2). A significant positive effect on cell proliferation was also observed in the shorter culture time (3 days) for Coll+CA. Both of these results are consistent with the cytocompatibility study (FIG. 1) in which cell viability after 72h was evaluated. Furthermore, there is a tendency for a slight slowdown in proliferation grown in the presence of medium conditioned with scaffolds modified with FA and GA. The scaffolds modified with phenolic acids slowed down the multiplication of bacteria in the bacterial medium, hence they were characterized by antibacterial properties (FIG. 3).

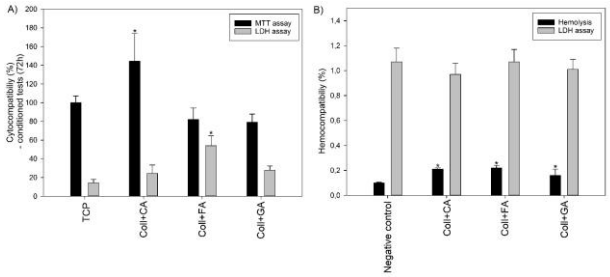

FIG. 1. The effect of developed scaffolds based on collagen modified by caffeic acid (CA), ferulic acid (FA), and gallic acid (GA) on cytocompatibility of hFOB 1.19 cells (expressed by MTT proliferation and lactate dehydrogenase release) after 72h of culture and hemocompatibility of human erythrocytes (expressed hemolysis rate and lactate dehydrogenase release) after 24h exposure to materials

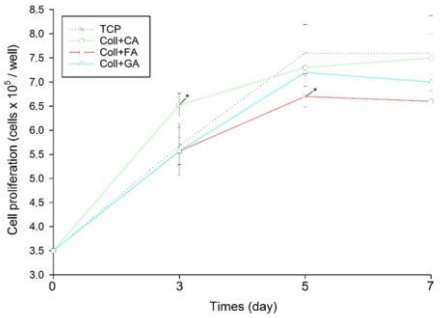

FIG. 2. The effect of developed scaffolds based on collagen modified by caffeic acid (CA), ferulic acid (FA), and gallic acid (GA) hFOB 1.19 cell proliferation

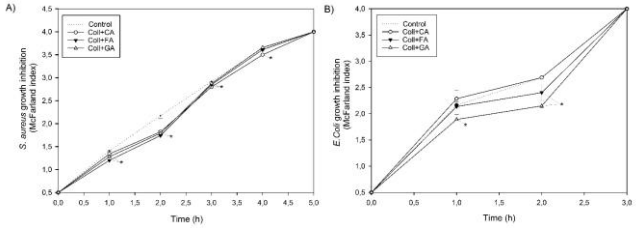

FIG. 3. The effect of developed scaffolds based on collagen with caffeic acid (CA), ferulic acid (FA), and gallic acid (GA) on bacterial growth inhibition determined by McFarland standard values specifying the number of selected bacteria during the incubation

Conclusions

All of the scaffolds were non-hemolytic and safe to be used in contact with blood. They are also cytocompatible. it may be assumed that collagen scaffolds based on caffeic acid and gallic acid are the most suitable for biomedical application purposes.

Acknowledgments

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αTCP-BASED BONE CEMENTS MODIFIED WITH GOLD DOPED BIOGLASS

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Introduction

Calcium phosphate based biocements are valuable bone substitution materials due to good bioactivity, ease of surgical handling and the highest biocompatibility among chemically bonded biomaterials. First material presenting bioactive properties was 45S5 Bioglass, invented by Larry Hench in 1969 and since then such materials have been attracting much attention in bone tissue regeneration as bone implants or modifiers of different biomaterials [1]. The idea of this study is to combine bioceramic bone cement based on α tricalcium phosphate (αTCP) and novel gold doped bioglass of unique composition in order to achieve new bone substitution material. The advantages of the designed material are among others higher level of bioactivity, injectability, ease of handling and appropriate time of setting. The addition of gold to the bioglass composition is promised to have a contrasting effect in magnetic resonance imaging, making the regenerated bone possible to be detected after resorption of substitute material.

Materials and Methods

In this study bioglasses from the P_2O_5 – CaO – $Ca(OH)_2$ – KF – TiO_2 system, doped with gold (500-2000 ppm) were obtained using melt quenching-technique. Gold was introduced via $HAuCl_4$-$3H_2O$. αTCP powder was synthesized via wet-chemical method and was used as setting agent. Distilled water and aqueous solution of Na_2HPO_4 were used as a liquid phase. Novel gold doped bioglass was added to the biocement system in the ammount of 10wt% or 20 wt%. Non-modified α tricalcium phosphate-based cement was used as a control material. Setting time was measured using Gillmore apparatus, compressive strength was tested using universal testing machine Instron 3345. Phase composition was checked using X-Ray Diffraction Method (XRD). Microstructure was observed using SEM (Phenom Pure). Bioactivity studies *in vitro* were performed in Simulated Body Fluid (SBF).

Results and Discussion

A significant difference between the times of setting of biocements without or with bioglass was not observed (TABLE 1).

TABLE 1. Setting times of obtained materials.

Sample	Composition	L/P	Initial setting time [min]	Final setting time [min]
S1	αTCP + 2% Na_2HPO_4(aq)	0.38	6.54	14.2
S2	αTCP + 10 % wt. BG1 + 2% Na_2HPO_4(aq)	0.35	6.25	12.50
S3	αTCP + 20 % wt. BG1 + 2% Na_2HPO_4(aq)	0.35	5.72	15.51
S4	αTCP + 10 % wt. BG2 + 2% Na_2HPO_4(aq)	0.35	6.10	13.75
S5	αTCP + 20 % wt. BG2 + 2% Na_2HPO_4(aq)	0.35	6.69	14.03
S6	αTCP + 10 % WT. BG3 + 2% Na_2HPO_4(aq)	0.35	4.35	11.94
S7	αTCP + 20 % wt. BG3 + 2% Na_2HPO_4(aq)	0.35	5.90	14.28
S8	αTCP + distilled water	0.38	7.57	22.24
S9	αTCP + 10 % wt. BG1 + distilled water	0.35	7.00	24.50

Biocement containing distilled water as liquid phase with the addition of bioglass shows more paste like consistency in comparison with the same biocement with disodium phosphate (Na_2HPO_4) solution (FIG. 1).

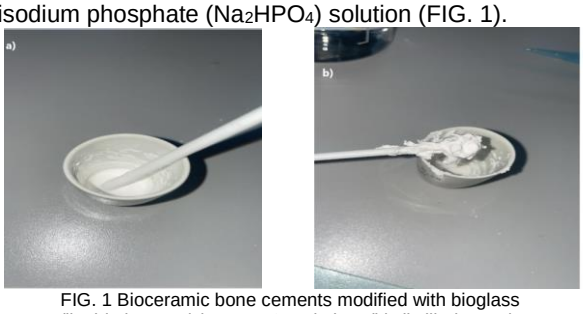

FIG. 1 Bioceramic bone cements modified with bioglass (liquid phases: (a) Na_2HPO_4 solution, (b) distilled water).

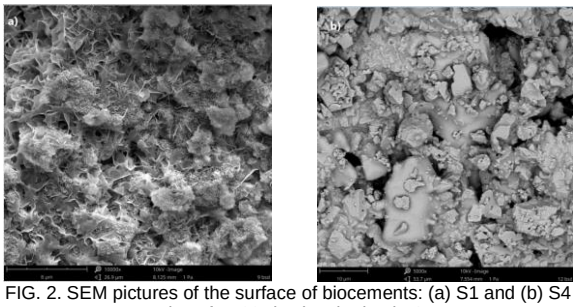

FIG. 2. SEM pictures of the surface of biocements: (a) S1 and (b) S4 samples after 28-day incubation in SBF.

The SEM observations of surface of the samples modified with bioglass did not show the formation of HAp layer in contrast to the biocement without modifier (FIG. 2). This type of materials may show similar effect in Kokubo test as βTCP based materials [2]. All obtained biocements modified with bioglass were injectable.

Conclusions

The difference between setting times of αTCP-based biocement without or with bioglass additive is not significant. It can be concluded that bioglass of the given composition does not affect the setting process of obtained materials.
The 28-day incubation in Simulated Body Fluid (SBF) of the cement samples with bioglass did not result in the formation of a hydroxyapatite layer on their surfaces. It can be stated that the Kokubo test is not an appropriate procedure for these types of material. Obtained biocements might be showing similar effect in Kokubo test as βTCP based materials.
The effects of liquefaction caused by bioglass addition makes the biocement materials more surgically handy and easily injectable.

Acknowledgments

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BIOMATERIALS MODIFIED WITH ANTIBACTERIAL AGENTS

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Introduction

Chemically bonded biomaterials based on calcium phosphates constitute a group of mouldable materials designed for bone tissue substitution and regeneration. These materials exhibit a range of beneficial characteristics such as biocompatibility, bioactivity as well as surgical handiness [1]. Biomaterials can be enriched with antibacterial properties by using various biologically active agents. Metallic nanoparticles and ions, such as silver, copper, and gold, exhibit inherent antimicrobial activity, that can effectively inhibit the growth and proliferation of a wide range of bacteria. The antimicrobial mechanisms of metallic nanoparticles and ions involve multiple pathways. They can induce oxidative stress by generating reactive oxygen species (ROS) within bacterial cells, leading to DNA damage, protein denaturation, and lipid peroxidation [2]. Furthermore, metallic nanoparticles can interact with bacterial cell walls and membranes, disrupting their integrity and causing leakage of intracellular components.
The aim of this work was to developed and examined chemically bonded biomaterials modified with antibacterial agents.

Materials and Methods

Chemically bonded biomaterials composed of α-tricalcium phosphate (α-TCP) and hybrid hydroxyapatite/chitosan granules, non-modified (HAP/CTS) and modified with biologically active agent (copper) (HAP/CTS/Cu), have been designed. The initial, highly reactive α-TCP powder, as well as hybrid granules, were prepared using the modified wet chemical method described previously [3-5]. The liquid phase of materials composed of citrus pectin (2.5 wt.%) and/or dihydrogen phosphate solution (1.0 wt.%). The chemical and phase composition (XRF, XRD), microstructure (SEM), setting times (Gillmore's apparatus), and compressive strength of the developed materials were investigated. Furthermore, the bioactive potential *in vitro* in SBF as well as antibacterial activity of the biomicroconcretes against *E. coli*, and *S. aureus* were evaluated (AATCC Test Method 100-2004).

Results and Discussion

Microstructural observations revealed the microporous nature of the calcium phosphate matrix and good adhesion at the matrix/granule interface. Due to the presence of a setting accelerator in the liquid phase, the obtained materials possessed acceptable setting times despite. The developed biomaterials exhibited compressive strength comparable to cancellous bone. Furthermore, the presence of citrus pectin allowed for reinforcement of materials from 8.4 ± 0.9 to the 14.7 ± 1.2 MPa, due to the various interactions in the structure of the materials. The *in vitro* tests in SBF revealed a high bioactive potential of the materials. Furthermore, the material of granules, due to the presence of chitosan and copper, exhibited antibacterial properties against the tested bacterial strains (FIG. 1).

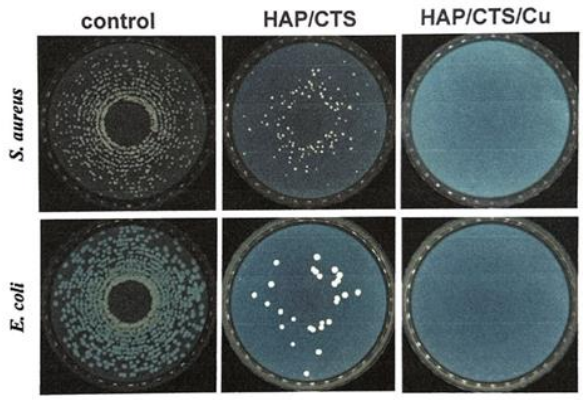

FIG. 1. Antibacterial activity of hydroxyapatite/chitosan-based materials non-modified and modified with copper.

Conclusions

Based on the obtained research results, it can be concluded that the developed bone substitutes with antimicrobial properties may be promising candidates preventing post-surgical infections in bone tissue applications. Further physicochemical and biological studies are necessary to obtain a comprehensive characterization of biomaterials.

Acknowledgments

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PLGA MEDICAL GRADE FILAMENT FOR PRODUCTION OF SHAPE MEMORY FDM 3D PRINTED SCAFFOLDS

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Introduction

Recent years, biodegradable shape-memory materials such as copolyesters of lactide and glycolide, have found application in the field of tissue engineering [1-2]. Changing the molar composition and polymer chain microstructure, it is possible to adjust their mechanical properties, degradation rate and shape memory effect to the particular needs. Using processing methods such as Fused Deposition Modelling (FDM) 3D printing, it is possible to obtain personalized, self-fitting medical implants in the form of cell scaffolds for regeneration of various types of tissues [3].
In the present work, a comparison of the shape memory effect for biodegradable polyester scaffolds depending on the copolymer molar composition, scaffold structure, deformation force direction and shape recovery temperature have been shown.

Materials and Methods

Different poly(L-lactide-co-glycolide) copolymers were synthesized by ring-opening copolymerization (ROP) of L-lactide and glycolide (both HUIZHOU Foryou Medical Devices Co., Ltd., China) in the presence of zirconium (IV) acetylacetonate initiator (Sigma Aldrich, Merck KGaA, Germany) in bulk [3]. Three molar compositions were obtained, differing in the ratio of lactidyl to glycolidyl units (85:15, 70:30 and 50:50). The synthesis was carried in PFA reactors at 130°C for 24 h, and then, the temperature was lowered to 115°C for 72 h. Monomer conversion, molar composition, as well as chain microstructure were determined by Nuclear Magnetic Resonance spectroscopy (Bruker Advance II Ultrashield Plus, USA). Gel Permeation Chromatography (Spectra Physics SP 8800 chromatograph, USA) was used to measure the average molar mass and Differential Scanning Calorimetry (Q2000 DSC, TA Instruments, USA) for determination the glass transition temperature (T_g). Obtained polymers were grinded (D-97877, Wanner, Germany) and processed by using twin-screw extruder (TSE 20/40, Brabender, Germany) in order to produce filaments with a diameter of 1.75±0,05 mm. The processing was carried out in the Clean Room laboratory, with ISO 8 air purity, in accordance to the PN-EN ISO 14644. FDM 3D printer (Dreamer, Flashforge, China) was utilized to obtain different structure porous scaffolds. The shape memory effect was tested using the strength testing machine, equipped with the temperature chamber (Model 4204, Instron, US) and a high-speed camera (SP-5000C-CXP4-C, JAI, Germany). The average recovery speed (V_r) and shape recovery ratio (R_r), were tested both after applying a compressive and stretching force, by incubating samples with an assigned temporary shape in a water bath at 37°C and 10°C above the T_g of the given copolyester. The morphology of scaffolds was assessed using scanning electron microscope (Quanta 250 FEG, FEI Company, US) and stereoscopic optical microscope (IPOS 810, Delta Optical, Poland). An *in vitro* cytotoxicity study was performed according to ISO 10993-5 standard, on human WI-38 (CCL-75) fibroblasts obtained from the American Type Culture Collection.

Results and Discussion

It was observed, that at the temperature of 37°C the average shape recovery speed increased with the increase of the content of glycolidyl units, which resulted from a T_g decrease. Measurements carried out at an elevated temperature, 10°C higher than the T_g of the copolymer of a given molar composition, resulted in the significant acceleration of V_r, which was particularly noticeable in the case of PLGA 85:15, because the onset point of the glass transition of this polymer was 51°C. This was due to lower mobility of the polymer chains at 37°C, than in the other cases, where the onset point temperature showed lower values. It was also observed that the average shape recovery speed was slightly higher for the grid structure of the samples, than the triangular geometry of layers, nevertheless, no significant changes in the value of R_r were ultimately observed. However, it turned out that the shape recovery ratio was lower for the compressed samples, than for the case of stretched samples, which resulted from the partial collapse and sticking together of the inner regions of scaffolds.
In the *in vitro* cytotoxicity studies, carried out using cell line, it was proved that both PLGA filaments and 3D printed scaffolds, also subjected to the β^- radiation sterilization process, did not show any toxic effect on fibroblasts.

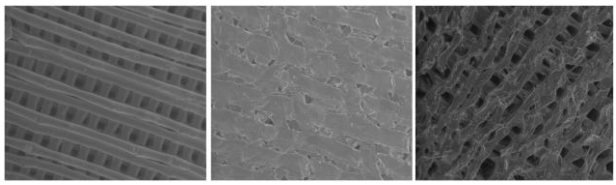

FIG. 1. SEM images (x250) showing the initial morphology of the exemplary PLGA 85:15 scaffold (A), the temporary shape after compression (B) and recovered structure due to shape memory effect (C).

Conclusions

Using biodegradable PLGA filaments, non-cytotoxic, porous scaffolds were obtained by 3D FDM printing. It was observed that not only the molar composition of PLGA copolymers, but also the internal structure of the scaffolds had an impact on the shape recovery ratio and the average recovery speed from the temporary shape to the original shape. Regardless of the direction of the deforming force, similar tendencies were observed in the tested materials. The main factor determining the V_r was the T_g of the copolyester and the geometry of the scaffold, while the R_r also depended on and the direction of the deformation force.

Acknowledgments

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MODIFICATION OF SIROLIMUS RELEASE KINETICS FROM ELECTROSPUN COPOLYESTER NONWOVENS USING DIFFERENT HYDROPHILICITY POLYURETHANE NANOFIBERS

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Introduction

Sirolimus is a drug that has found many applications. It is used both in the coating of coronary stents (prevention of restenosis), as an anti-rejection drug in organ transplantation, in the treatment of certain types of cancers and lung diseases. Due to its cytotoxic effect, it is important to maintain its concentration in the target tissue at an appropriate therapeutic level, which varies depending on the application. Polymer controlled drug release systems have become tools that allows the release of certain doses of the active substances directly to the target place in the body [1].
Present work presents a novel method of changing the kinetics of sirolimus release from biodegradable copolyester electrospun nonwoven, without changing the chemical and physical properties of the drug carrier.

Materials and Methods

Two biodegradable copolyesters with defined molar composition and chain microstructure were synthesized by ring-opening polymerization [2-3]. Poly(D,L-lactide-co-glycolide) and poly(Ɛ-caprolactone-co-trimethylene carbonate) were used as drug releasing matrix. Two-component nonwovens consisting of an interlacing of biodegradable copolyester microfibers releasing sirolimus and nanofibers of non-biodegradable poly(carbonate urethane) (PCU) modifier were produced using dual-jet electrospinning of polymer solutions. Three commercially available PCUs differing in hydrophilicity were used in the study. Using a time-varying feed rate of individual solutions, a gradient structure of the material was obtained, where the copolyester fraction was mainly accumulated at the surface region, and the PCU in the core. Obtained materials were incubated in the aqueous solution of phosphate buffered saline (PBS) at 37 °C for 24 weeks. Both the progress of degradation and erosion of the copolyester fraction as well as the rate of sirolimus release were studied. After incubation, samples were tested by analytical methods such as Nuclear Magnetic Resonance (NMR), Gel Permeation Chromatography (GPC), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) and High-Performance Liquid Chromatography (HPLC). Results were also compared with several mathematical kinetic models of drug release: zero-order release (0”), first-order release (1”), Higuchi (H), Hixon-Crowell (HC) and Korsmeyer-Peppas (KP).

Results and Discussion

Introduction of modifiers in the form of different hydrophilicity PCU nanofibers to the biodegradable copolyester nonwoven affected the affinity of the material to the aqueous environment during the incubation in PBS solution. This caused significant differences in the material ability to water absorption, which affected transport processes of water to the drug carrier as well as hydrolytic degradation products and sirolimus to the environment. It was observed that the increase of water uptake led to mechanism of surface degradation and erosion, while the hydrophobization of the material promoted processes in the bulk, accompanied by autocatalysis of the hydrolysis reaction, caused by poor removal of acidic copolyester degradation products to the incubation medium. Introduction of PCU nanofibers resulted in a decrease in the release rate at the initial stage in all cases, due to the extension of water diffusion path, resulting from the structure of nano- and microfibers interlacing. It was observed that the kinetics of the process was dependent of the difference in the water contact angle between the material of the drug carrier and modifier as well as the glass transition temperature of both components and the morphology of the matrix surface. Comparing the obtained empirical data with mathematical models of release kinetics, it was found that the main factors limiting the release rate were drug diffusion and sirolimus concentration gradient between the static layer at the matrix surface and the surrounding environment. After modification with PCU nanofibers, an increase in the fit of the results to the zero-order release kinetics was also observed. In most cases, the model that best matched the empirical data was the Korsmeyer-Peppas model.

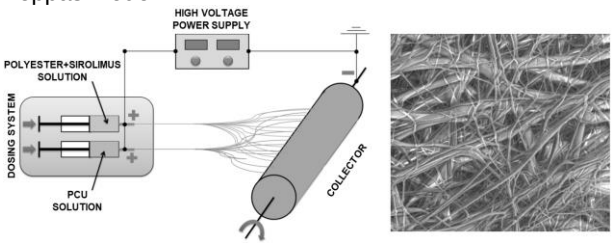

FIG. 1. Schematic representation of the dual-jet electrospinning (left) and SEM image of the resulting nonwoven (right).

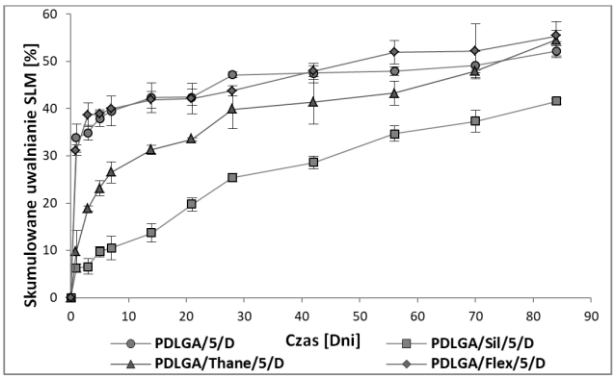

FIG. 2. Change in the release profile of sirolimus from PDLGA microfibers by using different hydrophilicity PCU-modifier nanofibers.

Conclusions

The introduction of PCU nanofibers into a copolyester nonwoven via dual-jet electrospinning, resulted in a change in the macroscopic hydrophilicity of the drug release system, which affected the transport processes of water, degradation products and sirolimus as well. As a result, a change in the release profile was obtained without changing the chemical and physical properties of the drug carrier.

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PDMS/NdFeB COMPOSITES SURFACE MODIFICATION USING SPIN-COATING METHOD

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Introduction

The PDMS/NdFeB composites are widely used in automatics, electronics, due to unique properties as smart material. For several years they have also been considered as potential materials for applications in biomedical engineering. Due to its possible applications in biomedical engineering, the surface is expected to prevent biofilm growth and exhibit antibacterial properties [1]. It has to be noticed that the material without additional preparation can cause adverse reactions and even be toxic.

Previous studies in the environment of 0.9% NaCl solution showed negative effects in the material and solution. Rust appeared on the material, while the liquid after incubation of the materials changed its properties in relation to the pure liquid [2].

Surface modification is one of the methods of changing the biological and physiochemical properties of the material. The functionalization of the surface should provide anti-corrosion protection, and protection against changes in surface properties during incubation, including reduction of the contact angle [3].

There are known ways to functionalize and change the properties of the PDMS surface, including modification with the addition of silane (APTES), plasma treatment, and UV radiation [4]. Unfortunately, due to the nature of the PDMS/NdFeB composite, surface modification using known methods is not sufficient.

The change of the properties is not the only problem regarding the PDMS/NdFeB materials. The mechanical or chemical protection against the release of NdFeB particles into the biological systems is also important [5]. Therefore, it seems necessary to prepare coatings that, on the one hand, will have a positive effect on the surface properties, and, on the other hand, will constitute a mechanical barrier against the particles release in a working environment.

The aim of the work was to experimentally verify the possibility of applying coatings with functional additives using the spin-coating method.

Materials and Methods

The PDMS-NdFeB composites were subjected to coating application. The concentration of the neodymium powder was 70wt.% of the total content of the composite. The materials were prepared by applying a composite solution to a flat surface and spreading uniformly over the surface using the spin-coating method. After the process the composite samples were dried in an incubator.

Solutions for coatings were prepared as follows: 1 part of PDMS was mixed with 1 part of a solution which consist of (a) chitosan and ferulic acid dissolved in 5% citric acid (PDMS-chit) [6] or (b) polycaprolactone solution in chloroform (PDMS-PCL) [7]. Each of the prepared solutions was stirred in a magnetic stirrer for 30 minutes in order to obtain a homogeneous solution.

The surface of the composites was covered with liquid coatings using the spin-coating method with various process parameters. The speed used for the process varied between 500 and 2000 rpm, changed every 500 rpm. The coating time was in the range of 10-30s, increased every 5s. After the spin-coating process the samples were dried in an incubator. The process was repeated on both sides of the thin composite.

The microscopic observations (CLSM, SEM) of the cross-section and surface of modified composites were performed to confirm the proper application of coating using spin-coating method. The water contact angle (θ) and thickness (h) of the obtained coatings were measured and analysed.

Results and Discussion

The prepared solutions were applied using the spin-coating process. After cross-linking, coatings with good adhesion to the surface of the hydrophobic PDMS/NdFeB composite were obtained.

The parameters of the spin-coating process affect the thickness of the obtained coatings. Setting the rotational speed to 1000 rpm and manipulating the time of coating deposition allowed to obtain comparable thickness of coatings from different solutions.

Water contact angle of PDMS-PCL coating ($\theta=82.6°\pm2.4°$) is similar to the contact angle of pure PDMS ($\theta=87.3°\pm1.8°$). For PDMS-chit coatings, the content of additives reduces the contact angle ($\theta=51.1°\pm2.9°$ for PDMS-chit 1%). The higher the chitosan concentration, the greater the contact angle reduction.

Conclusions

The paper verified the possibility of applying PDMS-based coatings to PDMS/NdFeB composites. As part of the research, the desired coatings were obtained on the composites, using spin-coating method. PDMS-based coatings are easy to apply on the hydrophobic composite surface.

The surface modification of PDMS/NdFeB composite by chitosan and polycaprolactone allows preparing materials for biomedical engineering applications. Other additives with functional properties may be considered in further research.

The knowledge obtained on a research basis is of practical importance for the use of this type of surface modification for various biomedical applications.

Acknowledgments

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BIODEGRADABLE NONWOVEN DRESSING CONTAINING PROPOLIS OBTAINED BY ELECTROSPINNING METHOD

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Introduction

The selection of appropriate dressing is crucial for the wound healing process. Wound healing can be a long-term process, often associated with infections, pain and unaesthetic scarring. Due to this, modern methods of treatment are sought to accelerate the repair of tissues as much as possible and minimize or eliminate complications [1–3]. The ideal dressing should provide a moist wound environment, accelerate re-epithelialization, accelerate angiogenesis and synthesis of connective tissue, allow gas exchange between the wound and the environment, provide optimal wound temperature to increase blood flow within it, pose barrier to infection, not adhere to the wound, minimize unpleasant smell, support the migration of leukocytes and enzymes, be transparent, allow observation of healing process, be sterile, be non-toxic and be non-allergic [4] Traditional dressings protect against contamination and mechanical damage of an injured tissue. Alternatives for standard dressings are advanced dressings containing a polymer with an incorporated active compound [5,6]. Previous studies on propolis-releasing nonwoven dressings made of poly(L-lactide-co-glycolide) copolymers (PLGA) shown promising results both in laboratory degradation and drug release tests, as well as in preliminary in vivo experiments, which were aimed at evaluating the therapeutic effectiveness of the obtained nonwoven fabrics on an animal model. Therefore, it was considered reasonable to perform further research aimed at producing nonwovens from copolymers poly(L-lactide-co-glycolide-co-trimethylene carbonate) (PLGATMC) and poly(L-lactide-co-ε-caprolactone) PLCL, as well as assessing their degradation process and the kinetics of active substance release.

The aim of this research was to obtain a biodegradable wound dressings made of poly(L-lactide-co-ε-caprolactone) 70/30 and poly(L-lactide-co-glycolide-co-trimethylene carbonate) 76/12/12 copolymers containing 5% and 10% od propolis (w/w), releasing active compound in a controlled manner to enable the adjustment of the release kinetics and the time of degradation of the carrier material to the needs of therapy.

Materials and Methods

Copolymers PLCL 70/30 and PLGATMC 76/12/12 were synthesized via the ring opening polymerization (ROP). The copolymerization was carried out at: PLGATMC: 120°C for 72 hours and PLCL: 150°C for 24 hours, in an argon atmosphere using zirconium acetylacetonate (IV) ($Zr[acac]_4$) as a non-toxic initiator.

Polymers (or polymers with 5% and 10% w/w of propolis) were dissolved in the mixture of solvents: chloroform and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in a volume ratio of 4:1 (v/v). Obtained solutions were used in the electrospinning process. The device was equipped with two high voltage power supplies. First, generating a positive electrical potential, was connected to the spinning nozzle and the second one, generating negative potential was connected to collector, in form of a rotating, steel mandrel.

Degradation and drug release studies of nonwovens obtained by the electrospinning process was carried out in phosphate buffered saline water solutions (PBS, pH 7.4) at 37°C for 84 days. Released active compound, the morphology of nonwovens, chemical composition changes of polymeric material during degradation process, weight loss and water absorption were determined.

Results and Discussion

FIG. 1 presents release of propolis from obtained electrospun polymer mats. After initial intensive release (which is beneficial for wound saturation with the active compound), the further release proceeded evenly.

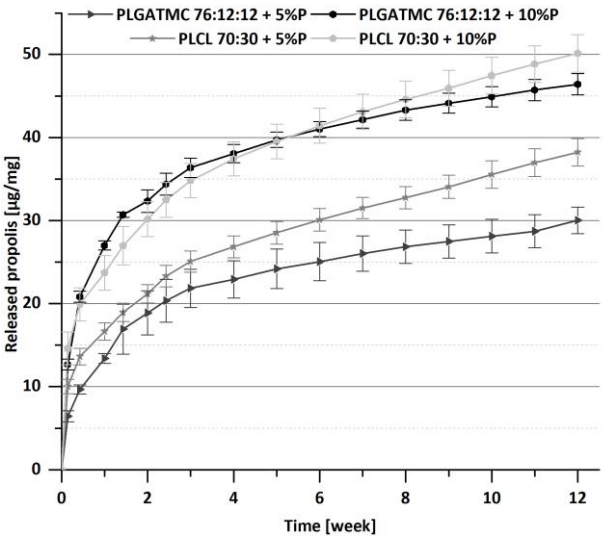

FIG. 1. Release of propolis from electrospun nonwovens. Results are presented as amount of released propolis [µg] per 1 mg of polymer mat ($\bar{x}$ ± SD, N = 3).

Presented results showed that changing the apitherapeutic content in the polymer matrix and polymer composition allowed to control the release profile of the apitherapeutic from the obtained material. Ability to control the propolis release profile allows to adjust the properties of obtained dressing to the requirements of the therapy.

Conclusions

The obtained biodegradable nonwovens release propolis in a controlled manner, and the active substance release profiles were different depending on the material used and the initial content of the active substance. The use of such a dressing material would avoid discomfort associated with the change of dressing or daily local administration of the drug substance, which makes them a potentially beneficial solution for wound treatment.

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CHITOSAN- GRAFT- POLY(ε-CAPROLACTONE) WITH PENDANT SCHIFF's GROUPS FOR COSMETIC FORMULATIONS MANUFACTURING

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Introduction

Recently, due to its wide range of applications and interesting properties, the attention of scientific researchers has been attracted by modifications of natural polymers, including such polysaccharides as chitosan, carrageenan, or alginates. Chitosan (CS) is an extremely useful, readily available bioactive polymer, which is characterized by the fact that it is renewable, non-toxic, edible, and above all biocompatibility.
In addition, the presence of two functional groups: hydroxyl and amine, gives the possibility of many different chemical and enzymatic modifications of CS, which is often used in the design and construction of systems for the immobilization and release of therapeutic compounds. Among the substituted biopolymers, particularly noteworthy are the Schiff bases obtained by the reactions of the free amino groups of CS with an active carbonyl compound such as aldehyde or ketone. CS derivatives, especially those synthesized via a Schiff base reaction, are very important due to their application characteristics. CS applications in cosmetics is a relatively recent issue, but more and more scientist are taking up this topic [1,2].

Materials and Methods

Low molecular weight chitosan was purchased from Aldrich Corp. (Steinheim, Germany) and subjected to a deacetylation process by Zhang's method [3]. As a result of the conducted reaction, the deacetylation degree of the reagent chitosan (100% -% DA) increased from 72% to 96%. ε-caprolactone was purchased from Acros Organics (Geel, Belgium) and purified using reduced-pressure vacuum distillation. 2-pyridinecarboxaldehyde; 4-formylbenzene-1,3-disulfonate; zirconium(IV) acetylacetonate; κ-carrageenan; glutaraldehyde; paraffin oil, Span 80 and α-tocopherol were purchased from Aldrich Corp., (Steinheim, Germany) and used as received. Organic solvents were purchased and used as received (Chempur, Piekary Slaskie, Poland). Synthetic membranes Strat-MTM were acquired from Merck Millipore (Darmstadt, Germany).

Synthesis and characterization of obtained products:
In the present study, in the first stage of the research, two chitosan–based Schiff–bases (CSBs) were obtained from the reaction of CS with different aldehydes i.e. 2-pyridinecarboxaldehyde and 4-formylbenzene-1,3-disulfonate. Then the purified CSBs were dissolved in DMSO and ε-caprolactone was added in a ratio of 1:8 GA: CL. The initiator of the conducted ε-caprolactone polymerization was the Zr(acac)4 in ratio 1: 1000. The reaction was carried out in a flask under argon for 72 hours at 80°C, then the product was filtered, washing it with chloroform, and then dried by lyophilisation.
The characterization of grafted products was measured based on 1 HNMR examination, at 600 MHz with the Advance II Bruker Ultrashield Plus Spectrometer (Bruker Corporation, Anaheim, CA, USA).

Blending: In the next step, compatible polymer blends of CSBs grafted by polycaprolactone with κ-carrageenan were formed in different weight ratios 70:30 and 50:50.
Forming the microparticles: To obtain the microparticles, the emulsion crosslinking method was used, which uses the reactive amino functional group of chitosan to crosslink with the aldehyde groups of the crosslinking agent. A water-in-oil (w/o) emulsion was prepared by emulsifying an aqueous solution of chitosan in an oil phase. The water droplets were stabilized with a surfactant (Span 80). The stable emulsion thus formed is cross-linked using a cross-linking agent, i.e. glutaraldehyde, to harden the droplets. The microspheres were filtered and washed several times with n-hexane, then alcohol, and dried.
Morphology and size distribution: Shape and surface evaluation of microspheres, was conducted by Tescan model VEGA 3SBU scanning electron microscope (SEM) (Tescan Orsay Holding, Brno, Czech Republic).
The release kinetics of α-tocopherol (vitamin E) from the microparticles was studied with high-performance liquid chromatography (HPLC) using a Smartline System (Knauer, Berlin, Germany) at 292 nm (UV/DAD).
The in vitro permeation studies were carried out in a Franz Cell system with a diffusion area of 1.767 cm2 and a capacity of 7 mL for the receptor medium, buffer pH 5. The Franz Cell system was maintained at a constant temperature of 36±0.5ºC, while the receptor medium was stirred constantly at 350 rpm. For the permeation studies, synthetic membranes Strat-MTM were used, and the donor part contained a typical basic cream base with the obtained microspheres carrier vitamin E. Aliquots of samples were collected at times 30min, 45min and 1, 2, 3, 4 and 6 h. The conditions were maintained with the replacement of the same volume of receptor medium. All collected samples were analysed by HPLC and quantification was obtained by the regression equation obtained from a standard curve.

Results and Discussion

Successfully, polymeric materials with interesting properties were obtained, which could be used to produce microparticles. The developed and applied method of forming allowed for the preparation of microparticles with a relatively regular shape, a fairly homogeneous surface and a relatively narrow diameter distribution with good reproducibility. The developed microparticles were filled with vitamin E and added to the cream base. Quantitative analysis of the release of the vitamin through the membrane, imitating human skin, showed a promising profile of its release/penetration, which gives hope for the development of a cream with anti-ageing properties.

Conclusions

Despite the small number of published research results in this field, it has been shown that chitosan derivatives can be successfully used as antibacterial microcarriers of bioactive compounds in various types of cosmetic formulations, such as creams and ointments.

Acknowledgments

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BIOMECHANICAL AND STRUCTURAL EVALUATION OF THE EFFECT OF GLYCEROL ON A RESORBABLE CHITOSAN/CELLULOSE SCAFFOLD FOR SKIN TISSUE ENGINEERING

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Introduction

Bioresorbable chitosan and cellulose are very well-known and attractive materials for skin wound applications and have shown some future perspectives that include both antibacterial and healing properties[1,2]. The weak biomechanical properties of these materials usually require crosslinking or blending with other synthetic polymers. The main objective of the study is to enhance the biomechanical properties and stability of porous chitosan/cellulose scaffolds in a simple one-step fabrication process without any additional crosslinking agents. We investigated the stability of manufactured scaffolds in medium and biocompatibility to verify their degradation time and effect of the degradation product on cells.

Materials and Methods

In the presented study, the effect of glycerol on the chitosan (Chit) scaffold mixed with either oxidized cellulose (Chit/OC), or carboxymethyl cellulose (Chit/CMC) was evaluated. The scaffolds were created by freeze dry process of biopolymer solutions with or without glycerol. The scaffolds with glycerol are as follows; Chit/OC/Glyc and Chit/CMC/Glyc.

The biomechanical properties were measured using RSA G2 dynamic mechanical analyser by monitoring tensile stress and strain under dry and hydrated conditions. The structural analyses were performed using scanning electron microscope (SEM) and software ImageJ to measure the porosity. The enzymatic degradation was provided in water-lysozyme solution and in cell culture medium by weighting the degraded scaffolds and calculating the mass loss over 21 days. The detection of degraded products has been measured with Fourier-transform infrared spectroscopy (FTIR). Wettability of scaffolds was evaluated by water contact angle measurement. To define the exact amount of swelling caused by water absorption at different time points, the excess water was removed, and the weight of dry and swelled sample was measured to calculate the swelling ratio. Finally, the cytotoxicity test with samples extracts were performed using fibroblasts cells.

Results and Discussion

Glycerol is a cheap and most commonly used plasticiser. It was already added as a biomechanical support for thin films and nanofibers in tissue engineering[3]. Incorporation of glycerol into the freeze-dried porous structures has shown a significant enhancement of viscoelastic properties. The elasticity of the material was 5-fold higher under dry conditions, while the strength of the material grew approximately 20-fold under hydrated conditions. The addition of glycerol significantly improved the enzymatic stability of the scaffolds and the stability in cell culture medium. Four times greater stability has been shown mainly for the Chit/CMC scaffold supplemented with glycerol, while it also reduced the amount of released degradation products, continuously measured over 21 days in enzymatic medium. Glycerol supplementation into porous scaffold could eliminate the utility of commonly used crosslinking agents. Furthermore, glycerol created a more hydrophobic character in the material and lowered swelling properties, while the porosity and pore sizes were not significantly influenced.

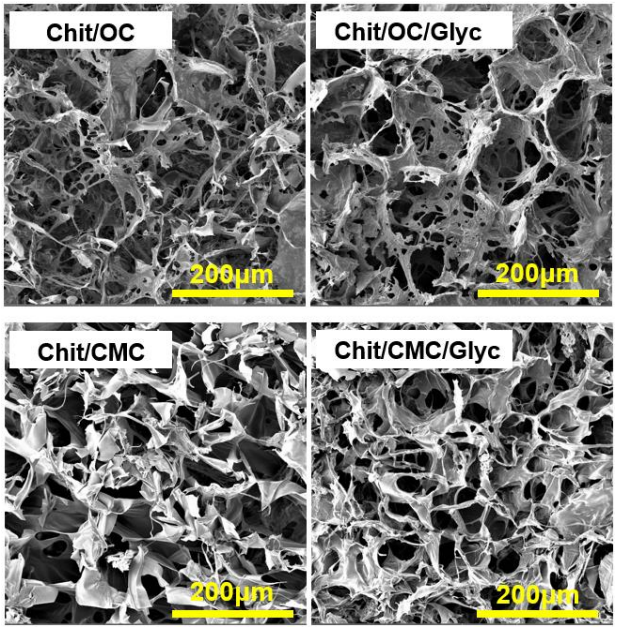

FIG. 1. SEM micrographs showing the structure of 4 types of chitosan/cellulose scaffolds.

Conclusions

The implementation of glycerol in freeze-dried scaffolds can strongly support elasticity and stiffness with sufficient scaffold stability and with a maintained porous architecture. The results are considered desirable and beneficial for future application in wound healing.

Acknowledgments

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BIOMATERIALS FOR WOUND HEALING: WHEY PROTEIN ISOLATE HYDROGELS CONTAINING CANNABIDIOL

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Introduction

Chronic wounds represent a global unmet medical need affecting around 18,000,000 individuals worldwide [1]. Multiple pathologies are present in a chronic wound environment in comparison to an acute wound such as an increase in inflammatory macrophages and mediators, matrix metalloproteinases, ROS, a microbial biofilm, and a sustained increase in pH circa pH9 [2,3]. There is a requirement for a treatment in the form of a dressing. However, a viable dressing requires properties such as wound exudate absorption and localised drug delivery. A new promising biomaterial, whey protein isolate (WPI) was previously demonstrated to load biologically active hydrophobic molecules [4]. Hydrophobic cannabidiol (CBD) has demonstrated potential in-vitro, in-vivo and ex-vivo to alleviate pathologies associated with a chronic wound environment [5]. Here, we combined both to WPI and CBD to create a hydrogel with potential as a wound dressing.

Materials and methods

WPI hydrogels were synthesised to 40% w/v with MilliQ. CBD was added to create WPI-CBD hydrogel variables to concentrations of 10, 20, 30, 40 and 50μM. The hydrogel solutions were degassed, gelation was heat induced at 70oC for 5 minutes. Swelling analysis was perform by introducing 1g WPI-CBD hydrogel to 5mL solutions of pH4, 7 and 9. The hydrogel samples were incubated for 5 days at 37oC. Release profiling utilised U.V. spectroscopy. The hydrogel samples were incubated in pH4, 7 or 9 for 5 days. At daily timepoints 100μL was removed and replaced with 100μL fresh pH solution. The solution was analysed with U.V. Vis spectroscopy at λ273nm. Statistical analysis utilised the ANOVA function in R-studio.

Results and discussion

The results for swelling and release analyses can be observed in FIG. 1. The maximum swelling potential was observed for the variable pH9 suggesting that when introduced to the high pH of the wound the hydrogels have to potential to absorb the maximum wound exudate. The addition of CBD to the WPI hydrogels had no detrimental effect on the swelling potential of the hydrogel. The maximum CBD release was observed at pH7 suggesting as the wound exudate is removed and pH is lowered the biological effect of the CBD could potentially be increased advancing wound healing.

Conclusion

The results demonstrate the potential of the WPI-CBD hydrogels to aid in wound healing. Firstly, suggested was the potential of the hydrogel to remove excess wound exudate. Secondly, was the potential of release of the CBD from the hydrogel enabling the CBD to perform the biological activity expressed in literature, specifically pathologies associated with a chronic wound state.

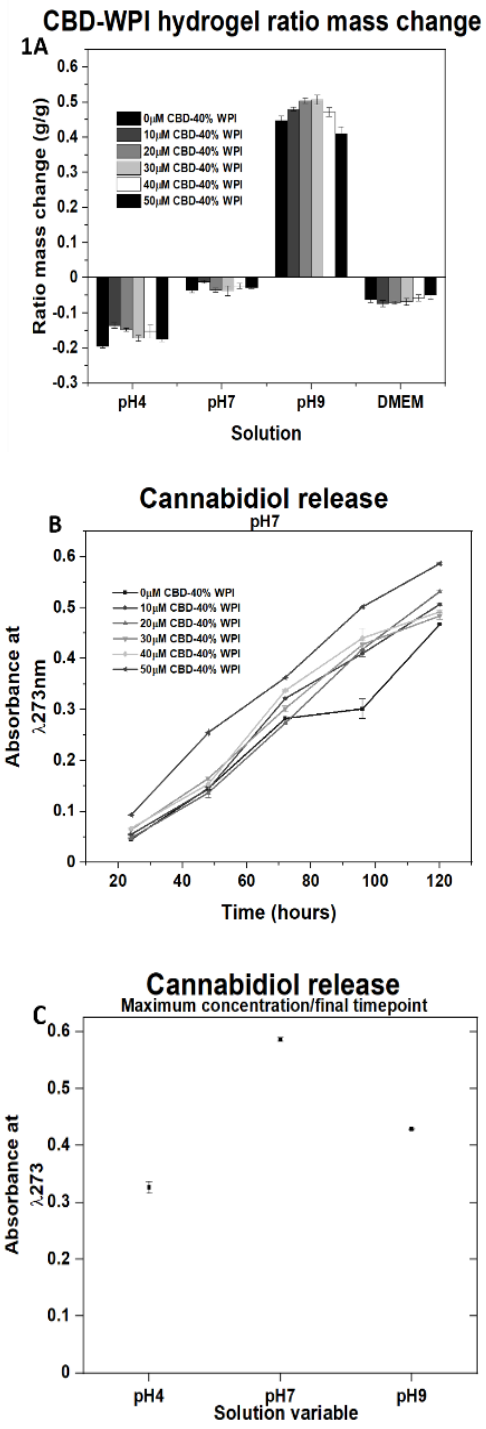

FIG. 1. (A-C); A) results of swelling analysis undertaken at different pH variables PH9 demonstrated the maximum swelling potential (n=15) (P≤0.05). B) example CBD release data acquired at pH7. C) maximum CBD concentration at the final timepoint demonstrating the maximum release for the pH7 variables (n=15) (P=≤0.05).

Acknowledgements

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COMPOSITE BASED ON POLYVINYL ALCOHOL, SODIUM ALGINATE, AND FUCOIDAN FOR WOUND-HEALING APPLICATIONS

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Introduction

Polymers are widely applied in biomedical field. Their film-forming properties are very useful for the fabrication of wound-healing materials. Sodium alginate and polyvinyl alcohol are applicable for wound healing separately and also as polymer blend. It has been shown that polymeric blend resulted in the improvement of chemical and mechanical properties [1]. Polysaccharides such as sodium alginate and fucoidan can also be applied in wound healing materials. Sodium alginate is preferred in wound healing applications due to its anionic nature [2]. Low molecular weight fucoidan has presented anti-inflammatory effects. Park et al. applied the low molecular weight of fucoidan for dermal wounds in the case of the rat model. It has been observed that it has anti-oxidant and anti-inflammatory properties. It has also shown growth-related effects [3]. In this study, we have analyzed the polymeric composites made from polyvinyl alcohol, sodium alginate, and fucoidan. The composites presented good mechanical strength, making them useful for potential wound-healing applications.

Materials and Methods

Polyvinyl alcohol (363065, CAS:9002-89-2, MW: 146,000-186,000), Sodium Alginate (CAS 9005-38-3, W201502, MW: 396) obtained from Sigma-Aldrich, Germany. Fucoidan (CAS 9072-19-9, MW:242.25, 98.35%) received from Arlington Hts, USA.

Solution preparation

Polyvinyl alcohol (PVA) 5% (w/v) in water, Sodium Alginate (NA) 5 % in water have been prepared in the proportion of 50:50. Fucoidan (FUC) 2%, 5%, and 10 % solution in water have been prepared.

Polymeric blend

The polymeric blend of PVA and NA has been prepared in 50:50 proportion and has been mixed with the Fucoidan 2%, 5%, and 10 %

Polymeric films preparation

Polymeric films of PVA 5%, NA 5%, polymeric blends like (PVA 5%+NA 5%, PVA 5%+NA 5%+FUC 2%, PVA 5%+NA 5%+FUC 5%, PVA 5%+NA 5%+FUC 10%) have been prepared by following the solving casting technique or solvent evaporation technique.

Mechanical Properties

The mechanical properties of polymeric films have been checked with the Zwick and Roell 0.5 mechanical properties testing machine.

FTIR Spectrum

Nicolet iS10 equipped with ATR device has been used to analyze the Fourier Transform Infrared (FTIR) spectrum.

Contact angle

Drop Shape Analyzer (DSA) 10, KRÜSS, Germany has been activated to record the contact angles of the polymeric films.

Results and Discussion

Polyvinyl alcohol and sodium alginate established stable polymeric films with the fucoidan. Polymeric films have shown good mechanical properties. FTIR analysis confirmed the homogenous mixing of the polymeric solutions in the form of functional groups. Contact angle measurements have been noted.

Conclusions

Polymeric films show good mechanical and chemical properties. The structure was confirmed by the FTIR spectrum of polymeric blend. Contact angle measurements showed that the surface hydrophilicity was changed after polymer blending. Surface properties have crucial role in the wound healing dressings properties.

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NITINOL SURFACE FUNCTIONALIZATION VIA HYBRID POLYMER MULTILAYER DEPOSITIONING

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Introduction

Shape memory NiTi alloys are unique biomaterials as only few of them exhibit shape memory phenomena. This is a key issue in biomedical applications where large reversible deformations are expected from devices, such as implants. Another important aspect to be taken into account is the biological response to a foreign body. Therefore, it is vital to protect the alloy surface against the influence of the biological environment and to improve its integration with the surrounding tissues. Hybrid polymer multilayers can play such a role. An example of a covering endowed with the structure and mechanical properties adapted to the substrate is presented in this work. Hybrid coverings were obtainedfrom the following layers: silicone, polycaprolactone and collagen.

Materials and Methods

Nitinol tapes (Kellogg's Research Labs) were used for the tests, with the top layer removed using sandpaper (800). A layer made of silicone (SI, Sylgard 184, Dow Chem Co) and polycaprolactone (PCL, 80 kDa, Sigma Aldrich) was applied to the cleaned surface. Silicone was administered with a brush to obtain a continuous layer serving as a barrier for physiological fluids, limiting their access to the metal surface. Polycaprolactone was applied via electrospinning as a fibrous layer serving as a scaffold for the rebuilding tissue. At the final stage, the composite was covered with collagen (Col, type 1 Sigma Aldrich) using the immersion method. Collagen was applied to increase the biological activity of the implant and improve the cellular response (FIG. 1). Microscopic methods, profilometry, X-ray diffraction (XRD) and FTIR spectroscopy were used to analyze the coatings.

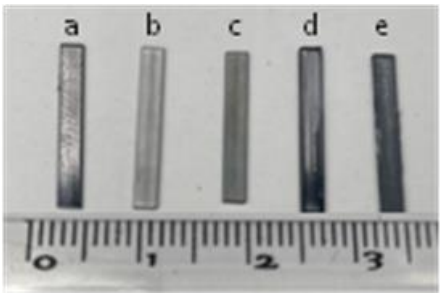

FIG. 1. NiTi strips: a) as received, b) after grinding, c) with electrospun PCL mesh, d) with silicon layer, e) with hybrid layer - silicon/ PCL mesh /collagen.

Results and Discussion

Microscopic observations confirmed the presence of polymer layers on the surface of nitinol tapes. The obtained layers had a uniform and continuous structure. The silicone layer was about 150 μm thick. No cracks or pores were observed. The polycaprolactone fibrous layer was much thinner, around a few micrometers, and was composed of fibers with a diameter of 0.5-2 μm (FIG. 2).

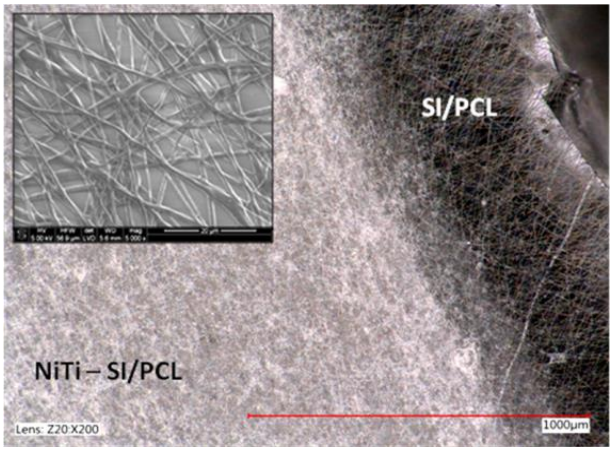

FIG. 2. Microscopic images of NiTi with SI/PCL multilayer (SEM and optical microscope).

The structure of the layers was examined using the X-ray GIXD method. The results are shown in FIG. 3. The phase analysis revealed the presence of a parent phase in the NiTi alloy substrate. However, the GIXD diffractograms confirmed the presence of crystalline Si and amorphous polycaprolactone.

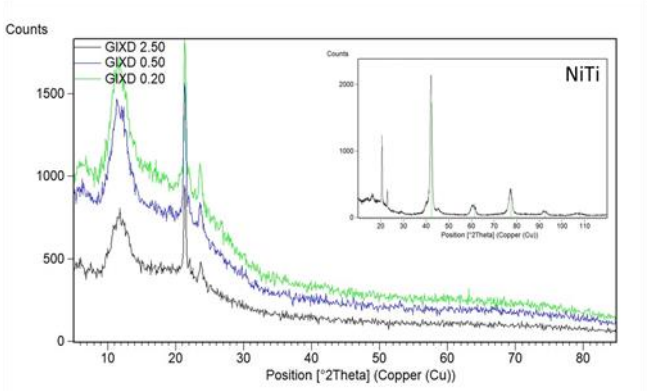

FIG. 3. GIXD diffractograms measured for the NiTi tape with the SI/PCL coating and the NiTi substrate only.

The collagen presence on the surface of the hybrid composites (SI/PCL/Col) was confirmed by the FTIR analysis.

Conclusions

The examined hybrid polymer multilayers, maintaining the assumed phase composition, adhered closely to the NiTi substrate, constituting an integral whole. The proposed composite with a diversified microstructure of the surface layer and the resulting multifunctional properties is a promising starting material. It can be can be utilized for implants in regenerative medicine with an extended period of use in the biological environment of the human body.

Acknowledgments

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HYBRID SOL–GEL COATINGS DOPED WITH SiO_2 AND hBN NANOPARTICLES APPLIED ON TITANIUM ALLOY

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Introduction

Titanium (Ti) or Ti alloys exhibits suitable mechanical properties and high biocompatibility as an osseointegrative implant materials [1]. Although Ti alloys has many benefits, it also has certain disadvantages. One of the major drawback is poor wear resistance [2,3]. Another obstacle is the metal ions release in contact with body fluids that have possible toxic effects to biological being [2]. The wreckage from wear and corrosion might react with bone tissues, leading to implant failure. Therefore, it is necessary to improve bioactivity and the tribological properties and prevent corrosion of titanium alloys through surface modification [3]. One of the prospective and dynamic trend in this field is the application of various protective layers [3]. Any of the forms applying coatings is the sol-gel technique which shows great potential as a means of corrosion protection allows the incorporation of antimicrobial ingredients into a pure silica matrix [3]. The main attractive point of this method is the ability to incorporation of antimicrobial components like boron nitride nanoparticles into a pure silica matrix [2,3]. Moreover, addition of silica nanoparticles into sol-gel coating has been reported to increase the barrier effect [3]. In this research, we have fabricated a hBNNP/SiO_2 coating for titanium implants by sol – gel method. The aim of this work was to investigate how deposited layers influence the microstructure and surface properties.

Materials and Methods

Two hybrid sols were prepared, one undoped and the other doped with hBN nanoparticles. The first sol was prepared at room temperature by mixing propanol, titanium (IV) isopropoxide (TIP), tetraethoxysilane (TEOS) and 3-(glycidyloxypropyl)trimethoxy silane (GPTMS) with a colloidal silica (SiO_2) suspension. After 30 minutes of stirring 0.2 ml of concentrated hydrochloric acid was added to 20 ml of solution as a catalyst. The molar ratio of the first hybrid sol was GPTMS/TEOS/TIP/SiO_2 = 0.33/0.6/0.05/0.02 and it was denoted as GTTSi. The second sol was prepared following the same process, but incorporating hBNNPs instead of SiO_2 suspension. Boron nitride nanoparticles dissolved in propanol, was agitated ultrasonically for 45 minutes and added before addition of the catalyst (HCl). The molar ratio of the second sol was GPTMS/TEOS/TIP/hBNNPs = 0.325/0.6/0.05/0.025, denoted as GTTBN.

Samples was subjected to a two- or three-step cleaning procedure. Substrates were firstly ultrasonically agitated in acetone for 30 minutes and then in ethanol for the same period of time. In a final step half of the plates was etched using 5% HF acid. After each step samples were rinsed with deionized water. Coatings were the combination of GTTSi and GTTBN sols deposited on Ti6Al4V alloy plates by dip-coating at a two different withdrawal rate of v_1=1mm/s and v_2=50 mm/s. Before depositing the second layer the first layer was drying in ambient conditions for 24h and then stabilized by the two-step thermal treatment at 80°C for 10 min and 130°C for 15 min. The same procedure was followed after the second layer had been applied.

Microstructure of the prepared coatings were examined by scanning electron microscopy. The surface properties of the modified TiAlV substrates, i.e. roughness, wettability and surface energy, also were investigated.

Results and Discussion

The surface observations of the layers revealed that boron nitride particles were not distributed homogeneously (FIG. 1). The layers took on the morphology of the substrate. The thickness of the coatings are influenced by withdrawal speed and the method of substrate preparation. Samples that were acid-etched were less well coated. When analyzing the surface roughness, we observed that the modification of the TiAlV alloy with the sol–gel layers significantly rudimentary the samples surface smoothness. The increase in the Ra and Rq parameters was observed for all the tested layers in relation to the base alloy. The tests also confirmed an increase contact angle values.

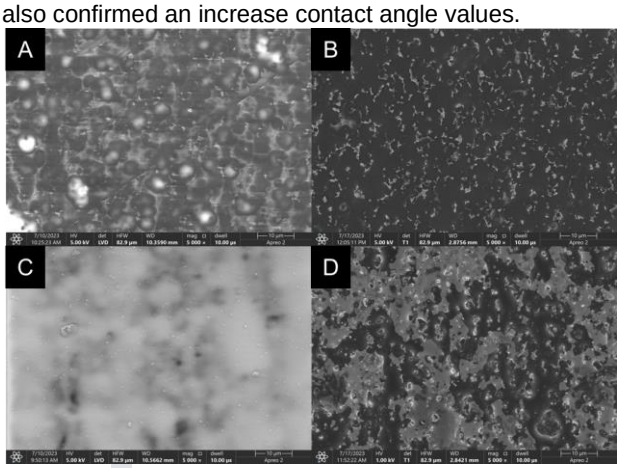

FIG. 1. SEM images of hBNNPs/SiO_2 coatings
A – withdrawal rate 1mm/s,
B – withdrawal rate 1mm/s, acid-etched sample,
C – withdrawal rate 50mm/s,
D – withdrawal rate 50mm/s, acid-etched sample.

Conclusions

The organic–inorganic sol–gel materials doped with hBNNPs were successfully synthesized and applied as coatings onto the Ti_6Al_4V plates. The Ti alloy layer modification changed the surface properties, such as wettability, surface energy, and roughness. Further research should focus on improving the homogeneity of the obtained layers.

Acknowledgments

This work was supported by program “Excellence Initiative – Research University” for the AGH University of Science and Technology, grant ID 1449 and ID 4089.

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MODIFICATION OF TiAlV ALLOYS WITH LAYER CONTAINING TiN NANOPARTICLES OBTAINED BY EPD METHOD

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Introduction

Over the last couple of years, veterinary medicine has changed its approach to animals' osseous injuries. Nowadays, more and more veterinary surgeons decide to employ different types of implants during orthopaedic treatment procedures. The most commonly used materials for bone implants are stainless steel and biocompatible titanium alloys [1,2]. Implantation has many advantages; however, it can also cause adverse reactions in the surrounding tissues. In order to prevent unfavourable reactions such as bacterial infections, collagen formation, or even necrosis [3], scientists are looking for solutions to improve both the mechanical and biological properties of the metallic implants. One of the solutions is to produce coatings that ensure antibacterial properties and provide protection against corrosion and scratches. These requirements can be achieved by applying an appropriate concentration of antibacterial nanoparticles in the coating composition. The latest research indicates that materials such as titanium nitride and its derivatives have significant potential in biomedical applications and are increasingly being investigated as biologically active additives [4,5]. In this study, the surface of a titanium alloy substrate was modified with the TiNNPs coating produced through the electrophoretic deposition process. The aim of this study was to access microstructure, chemical composition and surface properties of deposited coatings.

Materials and Methods

Coatings were developed by EPD on Ti6Al4V alloy plates. To prepare a final suspension for EPD process two different suspensions were prepared. The 1st suspension consisted of 0.125 g of medium molecular weight chitosan dissolved in mixture of 20 mL of distilled water and 0.9 mL of acetic acid. The 2nd suspension consisted of different amounts of TiN nanoparticles (to achieve 0.5 %, 1%, 1.5%, 2% TiN mass concentration in final suspensions), 5 mL of isopropyl alcohol and 25 mL of ethanol. The 1st and 2nd suspension were agitated ultrasonically for one hour and mixed. Each final suspension were than magnetically stirred for 24 hours. Before the deposition each final suspension was ultrasonically agitated for 2 minutes. Before the EPD process titanium alloy plates were firstly ultrasonically agitated in acetone for 30 minutes and than in ethanol for another 30 minutes. Just before deposition process titanium substrate was etched using 5% HF acid for 30 seconds than the plates were precisely rinsed in water. Electrophoretic deposition was conducted at voltage of 30 V and 1 minute deposition time. Thus obtained coatings were subjected to initial macroscopic observation as well as the SEM-EDS, confocal microscopy, XRD diffraction analysis, wettability and free energy surface tests in order to analyse the surface chemical compositions, microstructures as well as surface properties.

Results and Discussion

SEM observations confirmed the formation of a homogeneous coating of TiN nanoparticles for each tested concentrations. As the concentration of TiN nanoparticles increases, we can observe a corresponding increase in the density of the resulting layers (FIG. 1). The contact angle studies have revealed that the TiN chitosan coating changed the wettability of TiAlV alloy from hydrophilic to hydrophobic in all tested TiNNPs concentrations, which was confirmed by the obtained contact angle values in the range from 93º (1wt.%TiN) to 101º (1.5wt.%TiN). Layers containing nanoparticles are characterized by higher roughness parameters than pure titanium TiAlV alloy.

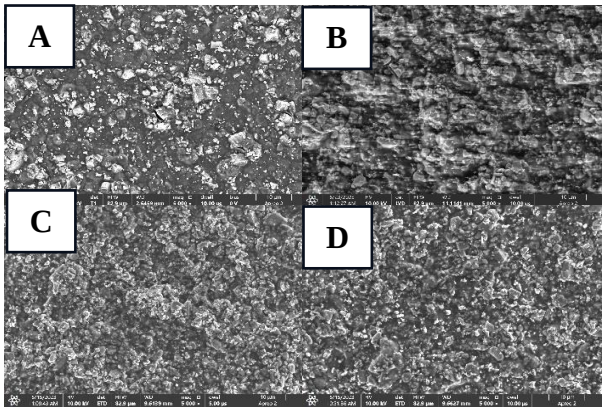

FIG. 2. SEM images of TiN/chitosan EPD coatings
A - 0.5wt.%TiNNPs, B - 1wt.%TiNNPs,
C - 1.5wt.%TiNNPs, D - 2wt.%TiNNPs.

Conclusions

The findings of this study lead to the conclusion that depositing homogeneous EPD coatings consisting of TiN nanoparticles and chitosan alters the tribological, physical and chemical properties of implant surfaces to varying degrees. Depending on the application of the implant, such changes may prove to be desirable. The deposited coatings demonstrate significant potential for further biological tests regarding their application in veterinary medicine.

Acknowledgments

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ENHANCED UV-PROTECTION IN CORNEAL REGENERATION: CARBON DOTS-MODIFIED CORE-SHELL FIBERS AS AN INNOVATIVE APPROACH

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Introduction

Prolonged UV light exposure of "unprotected" polymeric implants induces photo-oxidation which results in degradation and several adverse effects. These include mechanical deterioration, surface degradation, altered chemical structure (which affects biocompatibility) and leaching of additives (causing inflammation). This is a significant challenge for implants, particularly fibrous mats or nonwovens for ophthalmology, which undergo photodegradation upon UV exposure. Carbon dots are a potential solution due to their high UV absorption, good biocompatibility, low toxicity, and size that does not affect the transparency of the substrate [1,2].

The primary objective of this study was to fabricate electrospun core-shell fibers and subsequently modify them with carbon dots. The research aimed to evaluate the impact of these modifiers on the biological and optical properties of the nonwoven material, as well as its susceptibility to UV degradation. By understanding the carbon dots-modified fibrous materials, this research contributes to its potential applications in ophthalmic implants, addressing the issue of UV-induced degradation. Through this investigation, the study seeks to advance our understanding of carbon dots-modified fibrous materials offering a promising pathway for enhancing the performance and longevity of such biomedical devices.

Materials and Methods

Carbon dots (CDs) were prepared using the hydrothermal method. To obtain them, L-cysteine and NaOH were dissolved in water and homogenized with ultrasounds. Then the solution was transferred to the Teflon-sealed autoclave and heated at 150°C for 24h. In order to remove impurities, the obtained suspension was dialysed against 6KDa cellulose membrane for 3 days. To extend the absorption band, raw CDs were incubated in solutions of green tea, quercetin and a tryptophan-based peptide through 24h and then lyophilized. In the next step, core-shell fibers based on PCL-PVP were prepared with procedure described in the previous work [3]. To protect the PCL fibers, CDs were added to the core part. Characterisation of the CDs included measurements of a particle size distribution using the DLS method (Zetasizer Nano ZS, Malvern) and the UV-Vis absorbance spectrum UV-Vis, Shimazu). Microstructure of the nanofibers was observed with a scanning electron microscope (NOVA NanoSEM 200, FEI). Physicochemical properties (surface free energy, wettability) of the nonwoven was measured by a goniometer (DSA 25, Kruss). The light transmittance of the nonwoven was evaluated by spectrophotometry (UV-Vis equipped with an integrating sphere, Shimazu). Microbiological tests were conducted by the diffuse-disk method on the E.coli and S.aureus strains.

Results and Discussion

The CDs were characterized by a size distribution covering a range of 2-6 nm (FIG. 1A). The absorption bands of the raw carbon dots covered a wavelengths in a range of 190-350nm (the whole B UV and partially A UV) (FIG. 1B). Studies also shown the CDs bactericidal effect on the S. aureus strain (FIG. 1C). The applied electrospinning method allowed to obtain core-shell fibers with morphology known in the literature as a beads-on-string (FIG. 1D). The use of the outer layer of the shell during the electrospinning process limited the rate of evaporation of the core solvent and thus enabled obtaining fibers with small diameter and an unimodal and narrow size distribution in a range of 100-300nm (mean value 240nm). Due to combination of a hydrogel layer (PVP) and a presence of quercetin-modified CDs, the prepared nonwovens were characterized by high transparency in a Vis spectrum (>80%) and reduced light transmission in a UV spectrum (<20%) (FIG. 1E). The conducted microbiological tests showed that the encapsulation of carbon dots did not affect their microbiological activity, which was additionally enhanced by their functionalization with antibacterial substances (green tea extract, quercetin).

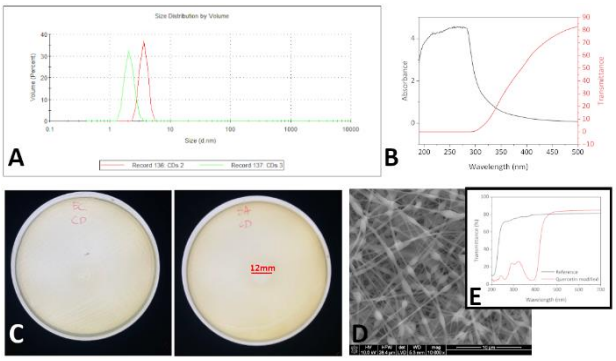

FIG. 2. CDs size distribution by volume (A), transmission and absorption spectra od CDs (B), Kirby-Bauer test results (C), nonwoven microstructure (D), light transmission through non-modified and quercetin-modified nonwoven (E).

Conclusions

The conducted research demonstrated the effectiveness of carbon dots as UV protective modifiers. Through additional functionalization, they effectively covered the entire UV range, providing enhanced protection against solar radiation. Moreover, the study revealed the potential of nonwovens as carriers of active additives. The employed manufacturing method (coaxial electrospinning) contributed to small and uniform fibers, crucial for optimal optical properties of the nonwoven material. The nonwovens exhibited remarkable properties, including high transparency and effective protection against UV radiation and bacteria. These findings indicate the promising potential of the prepared nonwovens as substrates for corneal regeneration.

Acknowledgments

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COLLAGEN-BASED FOILS AS POTENTIAL SUBSTRATES FOR *IN VITRO* CELL CULTURES – ANALYSIS OF SURFACE MORPHOLOGY AND PHYSICOCHEMICAL PROPERTIES

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Introduction

Biopolymer-based biomaterials are being widely investigated as substrates for *in vitro* cellular cultures as they possess a wide array of properties that can potentially mimic the native environment of cells in *in vivo* conditions. Among them, collagen-based scaffolds seem to be one of the most promising candidates, due to the fact that collagen is one of the main constituents of mammalian tissues. Its fibrillar structure and chemical properties not only help to maintain the proper shape of cells but also aid in their proliferation and differentiation [1,2]. Unfortunately, the processing of collagen in order to obtain a desired shape and morphology of a biomaterial is difficult. Due to this fact, solvents like HFIP are commonly used in substrate fabrication[3,4], which leaves the risk of cellular contact with unwanted toxic residues. Herein, we present materials obtained with the use of benign solvents.

The aim of the study was to fabricate materials based on type I collagen from benign solvent. The other aim was to modify collagen structure in mass with the use of glycerol and functionalized multi-walled carbon nanotubes to alter and potentially improve the morphology and physicochemical properties that might be important with the contact with cells in *in vitro* conditions.

Materials and Methods

Type I collagen (C9879), glycerol (G9012), sodium hydroxide (NaOH), hydrochloric acid (HCl), and fluoric acid (HF) were obtained from Sigma Aldrich. Dimethyl sulfoxide (DMSO) was supplied by Avantor. Roti®-CELL phosphate buffer saline (PBS) was bought from Carl Roth. Oxidized, multi-walled carbon nanotubes used in this study were obtained by following the protocol established by Benko et al.[5].

In brief, three 3% (m/V) collagen solutions were prepared by dissolving biopolymer in DMSO/PBS (5:1) solvent system, with the addition of 1M HCl and 5% HF. Mixtures were left on magnetic stirrers (4°C) until collagen was mostly dissolved. The samples were then processed with a tissue homogenizer. In the next step, pH of each solution was neutralized by a drop wise addition of NaOH. At this point, one mixture was modified in mass with glycerol (5% m/m_{col}), and another one with the addition of both glycerol (5% m/m_{col}) and carbon nanotubes (0,25% m/m_{col}). The last mixture was left unmodified and served as a control. All solutions were thoroughly mixed with the use of a vortex, poured on hexagonal weighing dishes. Samples were kept in the dryer at 40°C until fully dry. All collagen foils were stored at 4°C until further use.

The morphology of as-obtained materials was observed with the use of SEM (FEI Nova NanoSEM 200). Chemical analysis of surfaces was performed with the use of FTIR (Tensor 27, Bruker). The wettability of the samples was measured with the use of a goniometer (DSA25, Kruss). Mechanical properties were investigated with the use of Inspect Table universal testing machine (Hegewald-Peschke).

Results and Discussion

The morphology of the samples differed significantly depending on the side of the foils. “The upper” sides were characterized by uneven, porous topography, while the “bottom sides” were flattened and with noticeable less pores. Precipitated salt crystals, derived from PBS, were also abundantly visible on both sides. Chemical analysis of foils showed that the native collagen triple-helix structure was mostly preserved. However, significant differences between the “upper” and “bottom” sides in each of the samples were observed. Morphology and chemical properties translated into the wettability of foils. A water contact angle was significantly higher on the “bottom” sides, which were hydrophobic in contrary to the mostly hydrophilic “upper” sides. The influence of glycerol and carbon nanotubes on collagen structure was also observed. Lastly, a static tensile test revealed that the modifiers had an impact on the mechanical properties of the materials. Especially the addition of glycerol made the samples more elastic.

Conclusions

Pure and modified collagen-based foils were successfully obtained by following the as-described fabrication protocol. The surface properties of the materials varied significantly depending on the side of the sample, as well as the addition of certain modifiers to the collagen matrix. Due to the hydrophilic character, “the upper” side of the samples might be more favourable as a potential substrate for cell cultures. The differences in mechanical properties of collagen foils might translate into differences in cellular behaviour in in vitro cultures.

Acknowledgments

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OPTIMIZING PORE FORMATION IN DIALYSIS MEMBRANES: INVESTIGATION OF PREPARATION CONDITIONS

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Introduction

Membranes utilized in dialysis must possess specific properties necessary for efficient blood purification. These properties encompass an asymmetric morphology, consisting of a skin layer and a support layer, as well as a well-defined pore size ranging from 1 nm to 0.5 µm. The pore size plays a crucial role in selectively removing targeted molecules during the dialysis process, while the pore structure imparts mechanical strength, enabling the membrane to endure high pressure differentials encountered during therapy [1].

The manufacturing process significantly influences the pore morphology of dialysis membranes. Precipitation rates of a polymer solution govern the shaping of the pore structure. Subsequent immersion of the formed film in a nonsolvent bath results in the separation of the film into a polymer-rich phase forming the membrane matrix and a polymer-poor phase forming the pores. Several critical parameters come into play during this process, including the solvent evaporation time, phase inversion reaction temperature and the composition of the casting solution itself [2].

Understanding and optimizing these preparation conditions are of paramount importance for achieving membranes with desired properties for effective blood purification during dialysis. Control of the manufacturing parameters allows tailoring the membrane microstructure to meet the specific needs of the dialysis application, thus enhancing the efficiency and performance of dialysis membranes in clinical practice.

The aim of this study was to assess changes in morphology-related parameters: pore size distribution, pore shape, tortuosity and thickness of the skin layer. Tested variables concern changes in polymer casting solution by incorporation of carbon nanoadditives such as carbon nanotubes, graphite and graphene and changes in temperature of immersion bath.

Materials and Methods

Two sets of flat-sheet asymmetric membranes were fabricated using the phase inversion method, specifically the liquid-induced phase separation without solvent (NIPS) variation. The membranes were composed of a ternary system consisting of polysulfone (PSU) as the polymer, dimethylformamide (DMF) as the solvent, and water (W) as the non-solvent. Prior to the fabrication process, the membranes were tempered in ethanol (EtOH) for a period of 24 hours to optimize their properties. All components utilized in the membrane fabrication were sourced from commercial suppliers: PSU was obtained from Sigma-Aldrich, while DMF and EtOH were acquired from Avantor SA. For preparation of nanocomposites membrane there were used different shapes and sizes of carbon nanoform ie.; carbon nanotubes (Nanostructured & Amorphous Materials), graphene (NanoAmor US) and graphite (NanoAmor US). Each additive accounted consecutively for 0,5%wt., 1%wt., 2%wt. and 5%wt. of a sample.

Both kind of membrane sets: polymer (pure PSU membrane) and nanocomposite (with different carbon nanoforms as a additives) were characterized morphologically by scanning electron microscopy (SEM, Apreo 2). The pore size distribution of the membranes was obtained by mercury infusion porosimetry (MIP, PoreMaster 33). The effect of nanoadditives on the polymer structure in the final membranes was determined by differential scanning colorimetry studies (STA 449 F3 Jupiter Netzsch) in temperatures range from 20 °C to 650 °C. The performance of membranes was tested by determining the amount of BSA retained on the membrane by UV-Vis spectroscopy using the Exton reagent protein assay method (Shimadzu 1900i).

Results and Discussion

Characterization of the polymer membrane showed that temperature of the coagulation bath affects the shape, distribution and pore size of the membrane (FIG. 1a-c). With increasing temperature of coagulation bath the microstructure underwent changes, displaying an augmented presence of finger-like pores (with a diameter in the long axis direction longer than 30 um).

The largest macropores were obtained during precipitation of the membrane in a water bath at 30°C (FIG. 1c). Their finger-like shape provides better mechanical support for the entire membrane.

The incorporation of carbon nanoadditives and their concentration demonstrated the most significant impact on the skin layer's thickness and porosity. As the additive concentration in the casting solution increased, the skin layer became more compact, with a thickness reduction from 27 µm for 0.5%wt. CNT to 15 µm for 5%wt. CNT. Concurrently, the mean pore size of the skin pores decreased. The pore size distribution shows a unimodal character regardless of the carbon nanoadditives. All used carbon additives change the durability of the nanocomposite membrane and increase the degree of crystallinity of the matrix. Dynamic test with albumin solution established molecular weight cut-off at 60kDa.

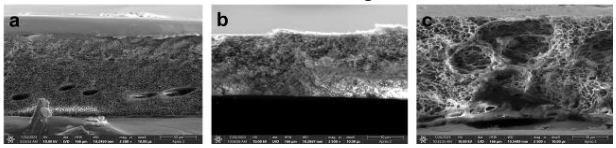

FIG. 1. Cross-section of pristine PSU membranes prepared in different precipitation temperatures a) 10°C b) 20°C c) 30°C. SEM micrograph, 2500x magnification.

Conclusions

A change in membrane preparation method results in a change in microstructure. By selecting specific conditions, we can model pore morphology that allow for more precise blood purification with a smaller loss of albumin.

Acknowledgments

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ANHYDRIDE-BASED COPOLYMERS OF SEBACIC ACID AND POLY(ETHYLENE GLYCOL) AS INHALABLE CARRIERS OF CURCUMIN

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Introduction

Fast biodegrading polymeric microparticles (MPs) may be used in dry powder inhalers (DPIs) to deliver drugs directly to the lungs, allowing to obtain the therapeutic effect at lower doses. Simultaneously, such an approach may reduce side effects, and minimalize the chances of developing resistance to the delivered therapeutics [1].
In the treatment of pulmonary infections with DPIs, the common problem is the need of using relatively big amount of MPs due to the limited drug loading capacity. This issue may possibly be solved by the use of quorum sensing inhibitors (QSi) e.g. curcumin (CU) – a common colouring factor in the food industry with. QSi prevent bacteria from creating biofilms, making them more sensitive to antibiotics [2].
In our previous study, we proposed poly(sebacic anhydride) (PSA) to obtain fast-degrading, nontoxic CU-loaded MPs with sufficient loading capacity [3]. Herein, we present our most recent work with the use of copolymer of PSA and poly(ethylene glycol) (PEG) for the same purpose. The aim was to evaluate the morphology, entrapment efficiency (EE) of CU in the MPs and to assess the influence of encapsulated CU on the degradation profile of the system.

Materials and Methods

Copolymers of PSA and PEG with M_w = 250 or 600 Da – namely PSAEG250 or PSAEG600, respectively – were obtained from sebacic acid and carboxyl terminated PEGs via polycondensation in a similar technique to the described earlier [3]. MPs were manufactured using oil-in-water (O/W) emulsification, where; O: PSAEG and CU dissolved in dichloromethane (DCM); W: water solution of poly(vinyl alcohol) (PVA). CU at the concentration of 20% CU:polymer ratio was dispersed in the solution using ultrasounds. MPs were obtained by adding the oil phase to the water phase, and then the organic solvent was evaporated under constant stirring. The MPs were washed 3 times in UHQ-water to get rid of the surfactant residues, and freeze-dried afterwards. MPs were observed using both optical and fluorescent microscopy. The EE and drug loading (DL) were evaluated by the fluorometric study of the MPs dissolved in dimethyl sulfoxide (DMSO) at excitation wavelength 485-412 nm and emission wavelength 590-510 nm. Degradation profiles of the MPs were analysed by suspending the powder in phosphate buffer saline (PBS, pH = 7.4) at the concentration of 1 mg/ml in Eppendorf tubes. The samples were kept in 37 °C for one week under constant gentle shaking. At chosen time points, the samples were removed, centrifuged, washed 3 times, and lyophilized to evaluate the mass loss of the MPs. Additionally, the pH of the supernatants was tested to monitor the progress in hydrolytic degradation of the samples.

Results and Discussion

Macroscopically, the obtained MPs were in the form of a yellow powder – the colour originated from the presence of CU (FIG. 1A). A closer examination with the optical microscope showed that they were spherical with a homogenic size distribution (FIG. 1B) – almost all MPs had diameters in range of 1-3 µm.

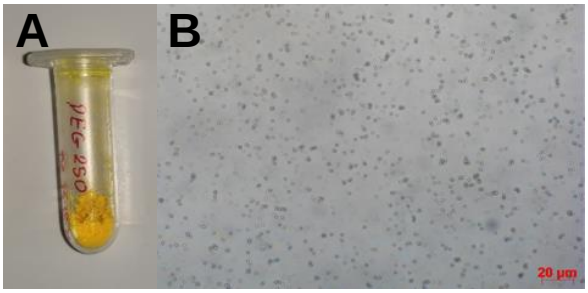

FIG. 1. Morphology of the PSAEG250 MPs loaded with CU: A – macroscopically, B – microscopically.

The EE values were assessed as 32.3 ± 0.6% and 21.9 ± 0.4% - resulting in DL values of 6.3 ± 0.1% and 4.6 ± 0.1% - for PSAEG250 and PSAEG600, respectively. Our improved DL evaluation method showed lower values than indicated earlier [3], however the use of MPs rather than supernatants allowed to exclude the potential bias from CU lost during the manufacturing process by, for example, changing containers with the samples.
During the incubation in PBS, the most rapid degradation occurred within the first 24 h. Over this time, the pH decreased to around 6.5 and 6.6, which was associated with mass loss of around 40% and 30%, for PSAEG250 and PSAEG600, respectively. After seven days of incubation, only 20-30% of the initial MPs were left, and the final pH of the solutions was slightly above 6. This indicates that the MPs degrade quickly in aqueous environment without the drastic effect on the pH. Such an observation suggests that the MPs would be able to release the CU in lungs before clearance mechanisms would remove them. Moreover, lack of the rapid changes in the pH is promising not to generate an inflammatory response of the host.

Conclusions

This study showed a potential of PSAEGs as CU carriers to deliver CU to the lungs. The EE and DL were satisfactory and the degradation profiles accurate for the purpose of the lung administration. PSAEG250 appeared to be better, as it showed a higher EE and DL, as well as it degraded slightly faster, which would lead to a more efficient drug release. However, biological studies are required to evaluate cellular response to the presented MPs.

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HYDROGEL DRESSINGS ENRICHED WITH CURCUMIN CARRIERS FOR THE TREATMENT OF DIABETIC FOOT ULCERS

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Introduction

Chronic conditions, such as diabetes contribute to chronic wounds, which are characterized by a longer healing time due to the prolonged inflammation phase [1]. Hydrogels provide a suitable aqueous environment and are commonly used to treat diabetic ulcers [2]. Moreover curcumin (CU) has been reported to inhibit the inflammatory response and to improve wound healing [1]. The aim of this work was to manufacture and characterize the properties of gellan gum (GG) based wound dressing enriched with poly(L–lactic–*co*–glycolic acid) (PLGA) nanoparticles (NPs) as CU delivery systems for the treatment of diabetic foot ulcers.

Materials and Methods

The NPs were manufactured from PLGA by a solvent evaporation method with oil–in–water emulsification. The oil phase was a 2% solution of PLGA in dichloromethane (DCM) with CU (CU:PLGA ratio of 1:20; 1:10 and 1:5) and the water phase was a 2% solution of polyvinyl alcohol (PVA). Oil phase was poured into water phase and homogenized with ultrasound for 3 min at 40% amplitude. The solvent was evaporated on a magnetic stirrer at 1000 rpm for 24 h. The prepared suspensions were centrifuged using 15000 rpm for 20 min at 4 °C. Particles were washed three times with UHQ–water and freeze dried for 24 h. The morphology and size of the NPs were characterised by scanning electron microscopy (SEM) and dynamic light scattering (DLS), respectively. Encapsulation efficiency of CU was evaluated in the supernatants obtained after the centrifugation using the fluorometric assay. Hydrogels were prepared as described earlier [3]. Briefly, equal volumes of crosslinking agent $CaCl_2$ (1% w/v) and MPs suspension (1 mg/ml) were added into a falcon containing dissolved GG, mixed on vortex, cast into a Petri dish and cooled down. Samples in the form of discs (12 mm in diameter and 4 mm in height) were cut and freeze dried for 48 h. Swelling and mass changes as a function of incubation in PBS were tested. The cytotoxicity of 1% and 0.5% extracts received after 24 h of samples incubation in Dulbecco Modified Eagle's Medium (DMEM) in contact with L929 fibroblasts (10 000 cells/well) was determined using resazurin-based metabolic activity assay and live/dead fluorescent staining.

Results and Discussion

The carriers were manufactured with 40% efficiency. NPs were round and homogeneous (FIG. 1). The addition of CU caused a yellow colour of the particles. The CU concentration of 5% and 10% w/v did not change the NPs diameters and they were 213 ± 9 nm and 194 ± 17 nm, respectively. The carriers with 20% w/v of the CU are characterised by about two times higher diameter size, which is 468 ± 25 nm. Encapsulation efficiency was 80% and drug loading was 9%. The manufactured NPs were homogeneously distributed in the hydrogel matrix as shown by SEM examination. Swelling capacity of the dressing prototypes was >2000%. Extracts obtained after 24 h of their incubation in the culture medium were not cytotoxic for the L929 cells at the concentrations of 1% and 0.5%. Cell viability was similar for each sample type.

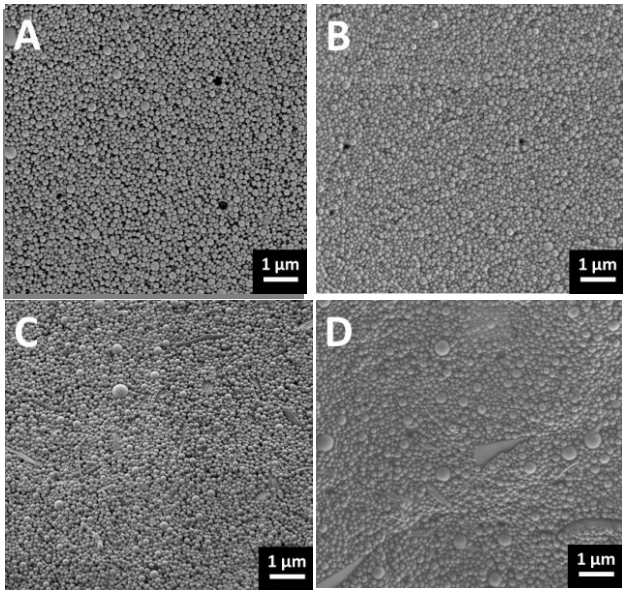

FIG. 1. Morphology of the PLGA nanoparticles A) empty; B) with 5% w/v CU; C) with 10% w/v CU; and D) with 20% w/v CU.

Conclusions

We have successfully manufactured NPs of defined size and satisfactory encapsulation efficiency of CU and suspended them homogeneously in the GG matrix. *In vitro* studies showed that dressings prototypes are not cytotoxic to L929 fibroblasts and can be considered for the treatment of diabetic foot ulcers. However, further studies such as evaluating CU release kinetics and more advanced *in vitro* studies with primary fibroblasts and keratinocytes should be conducted.

Acknowledgments

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PREPARATION BASED ON NATURAL POLYMERS WITH THE ADDITION OF SUBSTANCES REPELLANT TO TICKS

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Introduction

The Introduction should introduce the background to the work that has been carried out. It should contain citations to the key literature to support this rationale and should lead to a clearly stated hypothesis or set of objectives. Ticks (mainly of the genus *Ixodes*) are one of the most dangerous arachnids that can threaten us from early spring to the end of autumn. A tick bite can be very dangerous, because it can transmit very dangerous microorganisms: the etiological factor of Lyme disease (Latin borreliosis, morbus Lyme) - Lyme spirochetes (*Borrelia burgdorferi sensu stricto*, *Borrelia garinii and Borrelia afzelii*) and tick-borne encephalitis (TBE) by the KZM virus. The natural habitat of ticks is moist, mixed forests, but they can also be found in gardens, parks or clearings, in places with trees, shrubs or tall grass. These arachnids recognize their victims by the smell of sweat, changes in the ambient temperature and the concentration of carbon dioxide, and they react instantly by falling off a tree or sticking to the skin [1]. There are many preparations available on the market, for use directly on the skin in the form of sprays), spraying especially synthetic preparations, e.g. permethrin, cypermethrin or other pyrethroids (these are preparations harmful to human and animal health), as well as tablets and collars (for animals) [2]. Currently, there are no preparations available on the market based on natural polymers with the addition of effective repellants acting on ticks.

Materials and Methods

As part of the research work, active preparations (in liquid form) based on natural polymers with the addition of selected compositions of essential oils acting repellently on ticks were developed.
Solutions of natural polymers (chitosan salt with increased pH, aqueous solution of carboxymethyl cellulose and aqueous solution of sodium alginate with the trade name Manucol DH) were used to develop preparations with a potential repellent effect. The following commercially available surfactants were used: SLP, Tween 80, Olivem 1000, Poloxamer (184, 188, 407) and others, The oil phase consisted of essential oil compositions: tea tree oil, citronella, patchouli, clove and herbal aromas such as lavender, thyme, mint, oregano, lemon balm, rosemary, cinnamon, eucalyptus, geranium, lemongrass, marigold and others.
The research methodology is based on emulsification methods, i.e. creating an oil-in-water emulsion. The research methodology is illustrated in FIG. 1.
Evaluation of physicochemical properties (pH, conductivity), rheological properties (dynamic viscosity using a Brookfield viscometer), biological properties (susceptibility to biodegradation in the aquatic environment and ecotoxicity) and stability, using the centrifugal and thermal method of produced preparations. The emulsion systems produced were evaluated in terms of physicochemical properties (pH, conductivity), rheological properties (dynamic viscosity using a Brookfield viscometer), biological properties (susceptibility to biodegradation in the aquatic environment and ecotoxicity) and stability using the centrifuge and thermal.

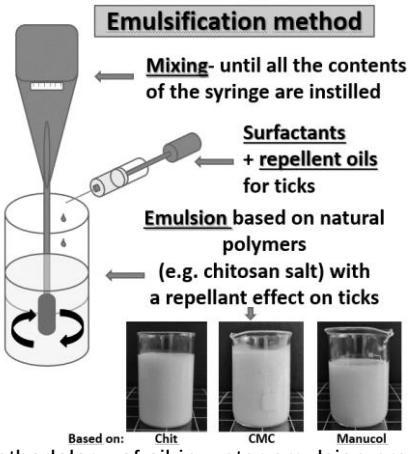

FIG. 1. Methodology of oil-in-water emulsion preparation.

Results and Discussion

TABLE 1 presents the results of the assessment of physico-chemical and rheological properties of emulsion systems.

TABLE 1. Physico-chemical and rheological properties of emulsion systems.

SAMPLES	pH (20 °C)	Viscosity, cP	Specific electrical conductivity, µS/cm
P-26/1	6,30	45,69	1410
P-26/2	6,30	53,02	1290
P-26/3	6,40	43,11	1390
P-26/4	6,34	55,66	1280
P-26/5	6,35	61,16	1250
P-26/6	6,44	40,38	1470
P-26/7	6,31	108,02	1990
P-26/8	6,36	140,06	2010
P-26/9	6,40	68,95	1810
1% aqueous manucol solution	7,39	29,46	-
1% aqueous CMC solution	7,42	31,19	-
1% aqueous Chitosan solution in 0,45% LA	6,47	50,78	-

Behavioral studies on ticks, biodegradability and ecotoxicity are in progress

Conclusions

The conclusions have to be based on the facts in evidence and should be limited to minimal speculation about the significance of the work.
1. Three polymer matrices were used to prepare the emulsion systems: Manucol - sodium alginate, sodium carboxymethyl cellulose (CMC) and chitosan lactate (Chit).
2. Research on the rheological properties of the produced polymer / repellent emulsion systems has shown that the value of dynamic viscosity [cP] and the electrical conductivity correlated with it is significantly influenced by the quantitative and qualitative composition of the produced polymer preparations.
3. The tested polymer / repellent emulsion systems showed very good stability at 25 °C for 4 weeks.
4. A form of arrangement has been developed that will be directly applied to vegetation surfaces to repel ticks.
5. In the next stage of the research, appropriate biological tests on ticks are to be carried out in a specialized research unit with the use of the selected emulsion system

Acknowledgments

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ANTIMICROBIAL CARBON NANOFIBERS FOR FACE MASKS AND RESPIRATORS

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Introduction

Carbon fibers have been the object of research in the context of medical applications for years. Previous reports in this area also contained controversial results. Currently, this material is proving to be increasingly useful in a number of new applications, including diagnostics and medical therapy, as well as in the construction of filters for face masks and respirators. Carbon nanofibers (CNF) in the form of non-wovens and different forms of flexible textile products may be particularly useful for this type of application [1]. Potential physical and chemical properties of CNF, important from the point of view of medical applications, can be modified in a wide range, both in the fabrication process and after the process. At present, an extremely topical problem in is finding new material solutions for protection against bacteria and viruses, which carry more and more threats to human health. This problem concerns medicine, both in the area of searching for new solutions related to broadly understood implantation materials with antibacterial surfaces, as well as coatings applied to surgical instruments, or hospital infrastructure equipment in general. Currently, in addition to research on the development of this kind of materials, research is focused on new, biologically active filtration materials. Airborne microorganisms negatively affect human health, cause or exacerbate infectious diseases such as influenza, pneumonia, corona virus, etc. The problem in otolaryngology is not only the so-called biological pollution of the air, but also all pollutants, the amount of which is constantly increasing and which are an inseparable component in climate change. Such pollutants are the cause of allergic rhinitis, both in children and adults, which may lead to other diseases such as sinusitis, ear infections or bronchial asthma. The use of activated carbon for the purification of liquid and gaseous media has been recognized for many years, however, as shown by the results of new research on CNF, this form of carbon opens up completely new possibilities in this aspect. Carbon material in the form of CNF allows the preparation of products with the desired surface to weight ratio, in the form of mats of various structures, and their further volume or surface modification may lead to the development of multifunctional filtration materials. As already shown, polymer nanofibers have recently become a particularly interesting material for filtration purposes, e.g. filter efficiency improves with decreasing fiber diameter, and in addition a high surface to weight ratio increases capture efficiency and improves other parameters related to surface dependent phenomena [2,3]. The advantage of CNF in these applications is due to their significantly high thermal and chemical resistance and electrical conductivity. The aim of our work was to develop carbon nanofibers in the form of mats with antibacterial properties as a potential material for the construction of air filters.

Materials and Methods

The subject of the study were carbon nanofibers, obtained in the process of carbonization (thermal treatment) of a polymer nanofibers made of polyacrylonitrile (PAN), which were carbon nanofiber precursor. PAN nanofibers, manufactured in the electrospinning process in the form of two-dimensional mats, were carbonized at 1000°C. The carbon mats (nonwovens) obtained in this way were then subjected to oxidation treatment, and two groups of CNF were prepared, which differed in the type of coating (titanium sol and silicon/titanium sol) applied by the sol-gel method. The precursor of the titanium sol was - $Ti(OC_3H_7)_4$, while the Ti/Si sol was prepared by combining the titanium sol with a silicon sol, based on the MTES, DMDES precursors. Both groups of CNFs, i.e., CNF-Ti, CNF-Ti/Si, as well as the as-received CNF nanofibers, before and after oxidative treatment, were characterized by spectroscopic (Raman, FTIR) and microscopic (SEM/EDS) methods. Next, tests of nanofibers in contact with bacteria were carried out. The tests were carried out in accordance with the standard; PN-EN ISO 20743 - Determination of antibacterial activity of textile products (E.coli and S.aureus).

Results and Discussion

Carbon nanofibers in the form of mats were composed of single CNFs with a diameter in the range of 140-250nm. The polymer coatings applied in the dip-coating process only slightly increased the diameters of individual nanofibers. Spectroscopic studies allowed the identification of chemical groups formed in the sol-gel process on the CNF surface. Raman spectroscopy showed the differences in the structure of the nanofibers and determined the types of defects occurring in the as-received CNFs and CNFs subjected to oxidative modification. The as-received carbon nanofibers, are characterized by a hydrophobic surface with a small amount of oxygen functional groups. Coating such nanofibers, e.g. with titanium or titanium/silicon sol, does not lead to the formation of antibacterial coatings on their surfaces. On the contrary, additional oxidation of the CNF surface leads to the formation of surface oxygen groups, thanks to which it was possible to cover the nanofibers with Ti/Si sol, giving the carbon mats antibacterial properties. CNF studies have shown that both the chemical composition of the sol used to modify the surface of the nanofiber and the chemical state of the CNF surface, as well as its structure and degree of defects, are the key parameters influencing the obtaining of antibacterial coatings on the carbon material.

Conclusions

The preliminary results of our research indicate that appropriate modification of CNF leads to the development of a material that can be used as a filtration material. The unique properties of CNF in terms of modifying their surface chemical state or in the whole volume is a way to obtain a new generation of materials with controlled biological activity, against viral and bacterial infections.

Acknowledgments

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NANOCOMPOSITE COATINGS WITH SUPERHYDROPHOBIC PROPERTIES FOR MEDICAL PURPOSES

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Introduction

A number of materials have been used in medical therapy and diagnostics, the properties of which must meet medical requirements. These materials should be biocompatible, i.e. the response they evoke in a living organism should be appropriate for a given application, consistent with the type of relevant therapy. In addition to broadly understood the term “biocompatibility”, one of the extremely desirable features of this type of materials should be resistance to the formation of bacterial biofilm on their surfaces.

This problem is also current in the field of laryngology, because it concerns not only implants, such as cochlear implants, middle ear prostheses, voice prostheses, osteosynthesis materials, but also e.g. tracheostomy tubes, various types of bioelectrodes, biosensors and surgical tools. The formation of biofilm on the surfaces of medical materials is a complex phenomenon, starting with the adhesion of bacteria, which then produce a durable matrix (EPS), accelerating further growth, maturation and propagation of the biofilm over the entire surface of the biomaterial. As a result of such phenomena, there are a number of negative effects including perioperative infections or the need for revision operations of medical devices [1].

This results in the need to significantly increase patient care and the development of comorbidities. Various strategies for producing antimicrobial surfaces are known, e.g. using metal-based materials such as titanium, silver and copper, using coatings with organic molecules e.g., organosilanes or producing surfaces with drug release systems that contain various types of antibiotics or other antimicrobial drugs. Nevertheless, the use of biocidal coatings may be associated with a negative phenomenon, which is the formation of significant amounts of dead bacteria on the surface of biomaterials, which can also lead to perioperative infections.

Therefore, research that, on the one hand, consists in analyzing the phenomena accompanying the adhesion of bacteria to the surface of the material, and on the other, analyzes the physical parameters of the surface with anti-adhesive properties, seems to be extremely valuable. As it is known, superhydrophobic surfaces are a new trend in the search for effective solutions in the field of antibacterial materials [2,3].

The aim of our work is research on methods of manufacturing superhydrophobic coatings that prevent bacterial adhesion.

Materials and Methods

Nanocomposite coatings were made on the surface of austenitic steel. Siloxane sol and silica nanoparticles were used to obtain the coatings. Nanosilica with a grain size of 5-10 nm and 30-40 nm from Aldrich was used. Sol solution was prepared by the catalytic hydrolysis of the siloxane precursors, i.e. methyltrimethoxysilane (MTES, ≥98%, Sigma-Aldrich, USA) and dimethyldiethoxysilane (DMDES, ≥97%, Sigma-Aldrich, USA). coatings were made using three different methods i.e., dip-coating, EPD –sequence and EPD-co-deposition. The coatings were dried at 70°C, then subjected to microscopic (SEM, AFM) and spectroscopic (FTIR) measurements. Moreover, the contact angle was also measured. Selected samples were analyzed *in vitro* in contact with cells (CCL fibroblasts -110, MTT assay). Bioactivity assay (SBF) was performed.

Results and Discussion

The results of the study allowed to determine the correlation between the composition of the suspension obtained from siloxane sol and silica nanoparticles, the manufacture way and the values of the contact angle. The studied coatings, regardless of the method used and the type of suspension used to obtain them, were characterized by hydrophobic properties (angle values in the range of 120° to approx. 160°) (FIG. 1).

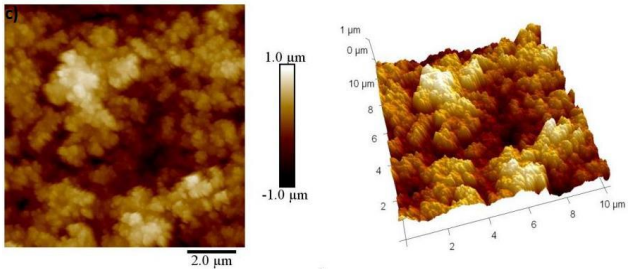

FIG. 1. AFM image of nanocomposite coating on a metal surface produced in the EPD process.

Superhydrophobic properties have been demonstrated in case of coatings that were prepared from suspensions containing silica nanoparticles (30-40nm) by means of the EPD co-deposition process.

Therefore these materials can be considered bacteriostatic and have anti-adhesive properties against bacteria. The coatings obtained as part of the research are not cytotoxic and display bioactive properties. As it is known, siloxane-based coatings are characterized by durability and resistance to degradation as well as good adhesion to the substrate [4].

However, their modification with nanocrystals significantly increases their functionality in the field of medical applications

Conclusions

Based on the obtained results, it can be concluded that the modification of polysiloxanes with ceramic nanoparticles (SiO2) is a way to obtain superhydrophobic materials that are not cytotoxic, bioactive, and according to the current state of knowledge, should protect medical materials against the formation of bacterial biofilm on their surface [3,5].

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CELL PROLIFERATION ABILITY DEPENDING ON CELL TYPE AND 3D PRINTING BIOINK COMPONENTS SOLVENTS

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Introduction

The development of hydrogel bioinks and 3D bioprinting technologies provides promising opportunities to imitate the architecture of damaged native tissues and bionically manufacture organ substitutes [1][2]. Numerous studies in the fields of medicine, dermatology and cosmetology are being carried out to enable the use of the primary cells in 3D models [3]. The selection of an appropriate hydrogel matrix for 3D cell culture is crucial for the proper proliferation and multiplication of cells to form a tissue structure. The unique properties of hydrogel materials ensure preparation of bioinks that meet the requirements of immortalized and primary cell lines [4]. Currently, beyond collagen, most widely used component of hydrogel bioinks for soft tissue scaffolds is sodium alginate [1][5]. Because of the lack in its structure of cell-interacting domains, this polysaccharide is often used in combination with gelatin, which has bioactive properties [3]. Apart from the composition, an important aspect in preparation of bioinks is the selection of an appropriate solvent for polymer solution. Our previous results indicate that the highest viability of cells contained in 3D printouts can be ensured by using culture medium as a solvent [6]. The aim of this study is the comparative analysis of the viability and proliferation of primary keratinocytes and immortalized fibroblasts contained in sodium alginate- and gelatin-based hydrogel 3D printouts. The research also includes an assessment of the effect of using different culture media to prepare the polymer compositions on the physicochemical and mechanical properties of hydrogel materials.

Materials and Methods

The initial stage of the study was to develop the method of isolation of the primary keratinocyte cells from foreskin tissues harvested from patients. Comparatively, for experiments an immortalized NIH/3T3 fibroblast cell line was used. Hydrogel material compositions based on 2% w/v sodium alginate and 9% w/v gelatin from pig skin type B were used. The powders were sterilized by UV light for 1 hour. Separate polymer solutions were prepared in DMEM – culture media dedicated for the fibroblasts (2A9G DMEM) and KBM-Gold – used for the keratinocytes (2A9G KBM-Gold). Analysis of the rheological properties and printability of prepared hydrogel compositions were carried out. Firstly, to obtain the highest viability of cells before the 3D bioprinting process, optimization of the method of combining cell suspensions with polymer solutions was worked out. Subsequently, bioinks containing $1x10^6$ cells/ml were used for 3D bioprinting of tubular structures. Printouts were crosslinked with 1% $CaCl_2$ solution for 10 minutes and incubated in culture media dedicated to individual cell lines for 0, 1, 4 and 7 days. After each incubation period, a Live/Dead test was carried out. In order to evaluate physicochemical and mechanical properties of prepared hydrogels, polymer solutions were poured into the Petri dishes, crosslinked with 1% $CaCl_2$ and divided into cylindrical samples. Samples were subjected to chemical structure analysis by FTIR spectroscopy, a static compression test and a swelling ratio analysis.

Results and Discussion

The results of the rheological properties analysis of the polymer solutions indicate that the 2A9G KBM-Gold is characterised by a higher viscosity. Mixing the polymer solution with the biological material does not significantly decrease the viability of cells. However, the process of 3D bioprinting and crosslinking of the printouts may result in a decrease of viability of the primary cells. After the 3D bioprinting process the viability of keratinocytes contained in the printouts equals 46.3 ± 13.9%, while the viability of fibroblast cells is 94.8±0.7%. During incubation of the printouts the viability of the keratinocytes progressively decreases. Also a dynamic degradation process of the printouts prepared in KBM-Gold is observed. It leads to their complete disintegration after 4 days. It may be a result of the composition of culture medium, which contains epinephrine, hydrocortisone and insulin. These components are able to combine with calcium ions and cause reduction of the hydrogel network structure durability. The decrease of primary keratinocytes viability may be also a result of a high concentration of calcium ions acting extracellularly during crosslinking and degradation of the material. During all incubation periods for 2A9G DMEM printouts a similar degradation process was not observed. The viability of the NIH/3T3 cells remained at approximate levels exceeding 80% at each timepoint. The 2A9G DMEM hydrogel samples are characterised also by Young's modulus of about 85 kPa, while KBM-Gold-based bioinks exhibit elastic modulus values below 100 kPa. After each of the swelling periods, 2A9G DMEM samples are characterised by a higher degree of swelling. Both materials show a slightly higher swelling capacity in PBS than in appropriate culture medium.

Conclusions

Primary keratinocyte cells are highly sensitive to external factors during the 3D bioprinting process and crosslinking of the 3D printouts. The type of cells introduced into the bioink plays a significant role in the selection of the hydrogel composition and printing parameters. The use of a universal bioink composition and identical printing parameters may lead to divergent results in terms of the ability and proliferation rate of cells contained in the hydrogel for direct bioprinting. The use of a medium dedicated to the cultivation of a specific cell line as a solvent for the preparation of a hydrogel significantly affects the rate of degradation of this material.

Acknowledgments

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FABRICATION AND CHARACTERIZATION OF STEARIC ACID BASED NANOPARTICLES LOADED WITH ANTIBACTERIAL PEPTIDE LL-37

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Introduction

Conventional treatment of bacterial infections is based on oral or intravenous administration of antibiotics, however since bacteria are able to quickly develop antibiotic resistance, such treatment is not always effective. What is more, as antibiotics are being overused (prescribed for non-bacterial infections or used for long period of time), bacterial resistance will grow making antibiotics not effective at all, leaving thousands of severely ill patients with no treatment options [1].

Novel methods for successful eradication of bacterial infections are needed. Antibacterial peptides (ABPs) are promising alternatives for antibiotics. ABPs are cationic molecules consisting of 12-45 amino acids. Their antibacterial efficacy have already been proven in different clinical trials. ABPs are able to hamper bacterial proliferation as effectively as antibiotics, whereby bacteria are not able to develop resistance for ABPs [2]. However, since ABPs are peptides in human body they are prone to inactivation by proteolytic enzymes or ions before reaching infected sites.

The aim of the following study is to develop novel delivery carriers for ABPs that will protect ABPs form premature degradation and allow their direct delivery to infected sites. We propose to use lipid nanoparticles as ABPs carriers, sine lipids are non-toxic, bioresorbable and can be used to encapsulate different types of molecules.

Materials and Methods

Lipid nanoparticles based on stearic acid (STE) were manufactured using oil-in-water emulsification/solvent evaporation method.

Firstly, appropriate solvent for STE was selected. Several organic solvents (i.e. hexane, chloroform, ethyl acetate, acetone) were tested. 50 mg of STE was dissolved in 2 ml of each solvent and then emulsified into 20 ml of 2% polyvinyl alcohol (PVA) solution via ultrasound homogenization (3 min, 40% amplitude). Upon complete evaporation of organic solvent, the obtained nanoparticles were purified and examined using atomic force microscopy (AFM).

Having selected the optimal solvent, several batches of nanoparticles were fabricated to evaluate the influence of different processing parameters (i.e. concentration and volume of organic phase, concentration of PVA, homogenization amplitude) nanoparticle size and polydispersity (determined by dynamic light scattering DLS). Cytotoxicity of unloaded nanoparticles was assessed in contact with L929 fibroblasts using metabolic activity tests (resazurin based) and crystal violet staining.

Finally, LL-37 antibacterial peptide was added to optimized nanoparticles. As LL-37 is not soluble in organic solvent, manufacturing method was adjusted (LL-37 was dissolved in water and emulsified into organic phase first, later manufacturing steps were unchanged).

The properties of LL-37-loaded nanoparticles were evaluated using AFM (morphology) and DLS (size and polydispersity). Efficacy of LL-37 encapsulation was determined by spectrofluorimetric method using *o*-phtaladehyde.

Results and Discussion

Among tested organic solvents only chloroform that is partially miscible with water allowed formation of uniform and spherical nanoparticles. In the case of immiscible hexane, the solvent could not evaporate from emulsion, while other solvents (characterized by higher miscibility with water) mixed with aqueous phase too quickly leading to precipitation of irregular, flake-like particles of STE. All further experiments were conducted using chloroform as a solvent for STE.

The most pronounced effect on particle size and polydispersity was observed for STE concentration in organic solvent and PVA concentration in external aqueous phase. In brief, the nanoparticles were smaller and more homogenous at lower concentration of the organic phase and higher concentration of PVA, which served as a surfactant. Nonetheless, it was not possible to obtain nanoparticles smaller than 300 nm.

Unloaded nanoparticles were tested *in vitro* in contact with L929 fibroblasts. It was found out that they had no cytotoxic effect on cells for up to 50 µg/ml, while LD50 was 107 ± 27 µg/ml.

The addition of LL-37 slightly influenced particle size, which decreased with increased LL-37 content, however the nanoparticles remained homogenous and spherical in shape. LL-37 was effectively encapsulated within nanoparticles, regardless of its initial concentration (encapsulation efficacy of 67% - 81%).

Conclusions

The developed method allowed formation of stearic acid-based nanoparticles characterized by spherical shape, low polydispersity and size in the range of 310 – 350 nm. The nanoparticles were non-cytotoxic and successful encapsulation of LL-37 peptide make them promising carriers for the treatment of bacterial infection. Further studies will evaluate their antibacterial efficacy and cytocompatibility.

Acknowledgments

The study was supported by Polish National Science Centre (project no. 2018/29/N/ST5/01543).

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FUNCTIONALIZATION OF POROUS ZIRCONIA SCAFFOLDS TO PREVENT IMPLANT-RELATED INFECTIONS

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Introduction

Bone is a connective tissue that plays significant roles in the living body, such as protecting all important organs. The self-healing properties bone tissue are limited and not all bone defects can be healed without surgical intervention. Transplantation is currently the gold standard for these types of defects [1]. Only in the United States, more than 100,000 implantations led to bone infections in 2004 [2]. Furthermore, the rate of treatment of implant-related infections failure is approximately 20% [3]. Due to a lack of tissue donors, there is an increasing need for alternate methods for treating bone tissue defects using scaffolds [1]. One of the most well-known, simple, and affordable methods of producing porous ceramic scaffolds is the polymer foam replication method. Different ceramic materials are being processed with this method, such as zirconium oxide (ZrO_2) [4]. Various techniques are used to alter the surface of zirconia ceramics, such as sandblasting, base or acid treatments, and polishing. Moreover, improved bioactivity can also be achieved by functionalizing the surface with bioactive materials [5]. In this study, we hypothesised that the calcium phosphate (CaP)-based bioactive layer with antibiotic-loaded nanoparticles (NPs) deposited on ZrO_2 substrates combines efficient bioactivity and bactericidal effects with superior mechanical characteristics. We believe that ZrO_2 scaffold coated with bioactive layer with antibacterial properties may provide a superior solution for alternative therapies for bone tissue treatment.

Materials and Methods

To produce porous zirconia scaffolds, the foam replication method was applied and two-step biomimetic co-precipitation was used to deposit the CaP layer. We investigated the microstructure and porosity of obtained scaffolds, likewise the morphology of the coating. Gentamicin sulphate was modified into hydrophobic gentamicin (gentAOT) by ion pairing method and encapsulated in poly(L-lactic-*co*-glycolic-acid) (PLGA). To produce PLGA NPs, the double emulsion method was applied. The encapsulation efficiency, drug loading, morphology, and size distribution were examined, and the drug release profile was investigated. Biological evaluation was performed with osteoblast-like MG-63 cells. Antimicrobial properties were verified with *Staphylococcus aureus*.

Results and Discussion

The shape, size, zeta potential as well as encapsulation efficiency (EE) and drug loading (DL) of NPs were examined. Both empty and loaded particles have spherical shape and the size around 200 nm. EE and DL of gentAOT loaded particles were sufficient and equal to 99 ± 1% and 9 ± 1%, respectively. Foam replication method is an efficient way to produce porous scaffolds with porosity over 90% and average pore size of around 250 µm. With the use of biomimetic co-precipitation, the CaP layer with introduced NPs was effectively deposited on the scaffolds. To investigate drug release, we incubated samples in PBS under dynamic conditions at 37°C. There was a significant difference between the concentration of gentamicin released from the scaffold compared to the free NPs. The release of antibiotic was faster, and there was more drug in the solution in the case of particles themselves. We performed tests with *S. aureus* in order to investigate the antibacterial effect of our scaffolds. Extracts of the scaffolds containing empty or loaded nanoparticles and the nanoparticles themselves were added to the bacteria suspension and cultured for 6 h. All investigated samples decreased the bacterial growth rate compared to the control. In addition, we performed the Kirby–Bauer test with the use of suspension of particles in PBS to confirm their antibacterial properties. The results show that the examined samples inhibited bacterial growth on agar plates. The cellular response of produced scaffolds with osteoblast-like MG-63 cells was examined to confirm their cytocompatibility. According to the results, cells proliferated well on all surfaces. They were well spread on the surface and the number of cells was growing with culture time.

Conclusions

This work aimed to functionalize the surface of zirconia in order to enhance its cytocompatibility with model bone cells and provide an antibacterial features. The modification of gentamicin sulphate was found to be an easy and effective method to improve the encapsulation efficiency without changing the size of NPs. With the use of the foam replication method, as well as biomimetic co-precipitation, we were able to obtain highly porous scaffolds with porosity greater than 90% and improved bioactivity. Samples are cytocompatible with MG-63 osteoblast-like cells and exhibit a satisfactory antimicrobial effect against *S. aureus*. The entire process of producing functionalized scaffolds is an efficient way to enhance the bioactivity of the zirconia surface and produce scaffolds with antibacterial properties.

Acknowledgments

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INFLUENCE OF GENTAMICIN AND MODIFIED GENTAMICIN ON *STAPHYLOCOCCUS AUREUS* BIOFILM FORMATION

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Introduction

Nowadays, several types of antibiotics are encapsulated in different carriers which are delivered directly to the infected place of the human body. The drug carriers are manufactured from different polymeric materials and many of them are hydrophobic in nature. However, several of the clinically used antibiotics are hydrophilic and thus are incompatible with hydrophobic polymer matrices, which results in low encapsulation effectiveness. On the other hand antibiotics can be modified by the hydrophobic ion-paring method [1].
The aim of this study was to modify gentamicin sulphate (Gent) with docusate sodium salt (AOT) and to compare the influence the Gent and GentAOT on *Staphylococcus aureus* biofilm formation.

Materials and Methods

Gent modification with AOT (both from Sigma Aldrich) was performed according to a method described previously [2]. Gent and Gent AOT were dissolved in UHQ-water and in dimethylosulphoxide (DMSO), respectivelt at five concentrations (1, 2, 4, 8 and 16 µg/ml).
S. aureus (American Type Culture Collection, ATCC 25923) biofilm formation was assessed. The bacteria were seeded in 24-well plates and incubated with antibiotics for 4 h. To 990 µl of broth, 10 µl of Gent or GentAOT solution was added. Additionally, one more sample was prepared by addition of 10 µl of Gent and 10 µl of DMSO to 980 µl of broth (Gent+DMSO). The minimum biofilm inhibition concentration (MBIC) was measured using the AlamarBlue method. The biofilm mass was measured by crystal violet absorption test.

Results and Discussion

Biofilm viability is presented in FIG. 1. MBIC for Gent was equal to 16 µg/ml, for Gent+DMSO – 2 µg/ml, and for GentAOT – 8 µg/ml. The biofilm mass reduction was similar for Gent+DMSO and GentAOT. For Gent biofilm mass reduction was lower. The results are presented in FIG. 2.

Conclusions

GentAOT has lower MBIC than Gent, but higher than Gent+DMSO. However, the biofilm mass reduction for GentAOT was similar to Gent+DMSO.

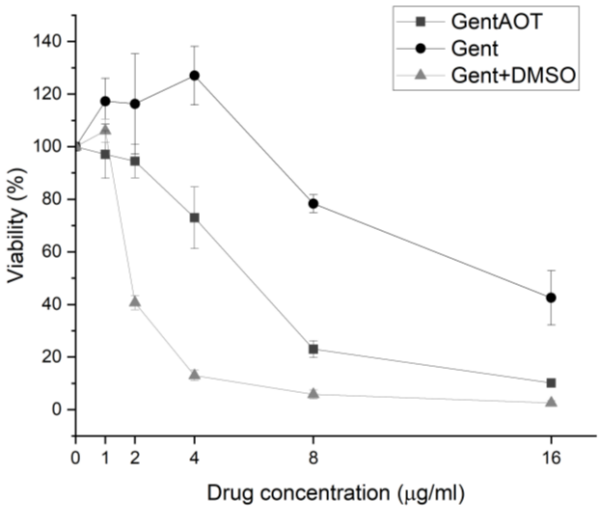

FIG. 1. Biofilm viability of *S. aureus* depending on drug concentration.

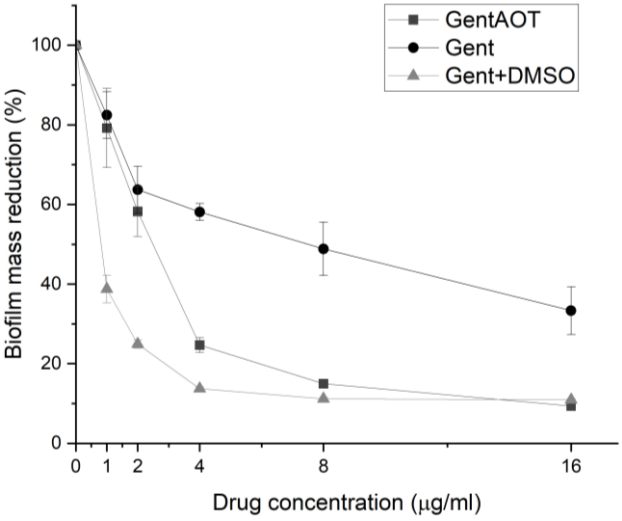

FIG. 2. Biofilm mass reduction depending on drug concentration.

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SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES SURFACE-STABILIZED WITH N-SUCCINYLATED CHITOSAN DERIVATIVE FOR TARGETED ANTI-CANCER THERAPY

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Introduction

Currently, newer and more effective methods are being sought to reduce mortality caused by cancer. The appearance of secondary tumors signals an advanced stage of cancer and is responsible for approximately 90% of all cancer-related deaths. Therefore, finding an effective method of capturing and eliminating so-called circulating tumor cells (CTCs), which are responsible for spreading the tumor to distant organs, is extremely important [1]. Nanotechnology comes to our aid, offering nanoparticulate systems with exceptional properties. Among them are the superparamagnetic iron oxide nanoparticles (SPION), widely used in biomedicine as MRI contrast agents or in magnetic hyperthermia. When surface-modified with a specific antibody they may also be used to magnetically capture cancer cells. Here we propose SPIONs surface-stabilized by a N-succinyl derivative of chitosan (NSCh), and further modified with anti-VCAM-1 antibody and phycocyanin.

Materials and Methods

N-succinylated chitosan was obtained by reacting chitosan with N-succinyl anhydride in aqueous 1 % solution of acetic acid for 8 h in room temperature. The product was isolated by precipitation with acetone and purified by dissolving in 3 % sodium bicarbonate and re-precipitating in acetone. For further purificantion the product was dissolved in water and precipitated with acetone two more times, then liophylized.
The ^{1}H NMR spectra of N-succinylated chitosan were obtained in D_2O using Bruker BioSpin spectrometer. The the substitution degree was calculated based on the signals from the methylene protons in succinyl group and signals from H3-H6 protons in chitosan structure. The degree of substitution was further confirmed by the colorimetric ninhydrin assay. ATR-FTIR spectra were obtained with Nicolet IS10 spectrometer (Thermo Fischer) equipped with ATR accessory. The size and zeta potential of NSCh-SPION were assessed by dynamic light scattering (DLS) using Zetasizer Nano-ZS and morphology was evaluated by STEM microscopy using SEM/FIB Quanta 3D 200i (FEI) microscope. Magnetic properties were determined by Vibrating Sample Magnetometer and Mössbauer spectroscopy. The attachment of the anti-VCAM 1 antibody and phycocyanin to the surface of the nanoparticles was confirmed using fluorescence spectroscopy using F-7000 fluorimeter (Hitachi).

Results and Discussion

Spectroscopic analysis confirmed the successful synthesis of NSCh. The obtained chitosan derivative showed good solubility in water, which was supported by the relatively high degree of substitution (55.49%) determined by the ninhydrin test and ^{1}HNMR spectroscopy. The obtained NSCh-SPION nanoparticles were spherical, colloidally stable, and exhibited excellent magnetic properties. Magnetic measurements confirmed the superparamagnetic character of the studied SPION systems. The formation of the anti-VCAM-1 - decorated NSCh-SPION system and the attachment of phycocyanin to the surface of these nanoparticles was also confirmed. Preliminary studies of the cytotoxicity of the obtained nanoparticulate systems were also performed using MTT assay.

Conclusions

We have successfully obtained a CTC-targeting nanoparticulate system utilizing SPIONs specifically engineered to capture CTCs. We conducted thorough studies of the physicochemical and magnetic properties of the obtained nanoparticles. Additionally, an initial evaluation of the biological properties of the synthesized system was also performed.

Acknowledgments

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THE INFLUENCE OF BIOACTIVE GLASSES VARYING IN COMPOSITION ON THE DUAL-CROSSLINKING OF INJECTABLE CHITOSAN HYDROGELS

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Introduction

The hydrogels based on dynamic covalent bonding have gained increasing attention in recent years in biomedical applications. This is primarily due to their unique properties, such as self-healing and injectability. In this study, silicate and borate bioactive glasses (BGs) were used as modifiers in the dynamic covalent chitosan-based hydrogels crosslinked with dextran dialdehyde. Both silicate and borate BGs can play a dual role in the obtained hydrogels – they serve a functional component, modifying hydrogel properties and provide additional multi-level crosslinking mechanisms. Two main mechanisms of crosslinking effect of BGs, resulting from the release of their degradation products can be identified. Firstly, boron and silicon provide dynamic covalent ester bonds. Secondly, calcium and sodium-mediated deprotonation of the NH_3^+ groups of chitosan enables the formation of additional imine bonds.

Materials and Methods

In this study injectable hydrogel materials based on chitosan, crosslinked with a dextran dialdehyde and different BGs were obtained. BGs such as high-calcium A2 (40 mol% SiO_2, 54 mol% CaO, 6 mol% P_2O_5) and 45S5 (45 mol% SiO_2, 24.5 mol% CaO, 24.5 mol% Na_2O, 6 mol% P_2O_5) silicate bioactive glasses and A2B40 (54 mol% CaO, 6 mol% P_2O_5, 40% B_2O_3) borate bioactive glass have a dual function – chitosan crosslinking agents and functional ingredients.

The aim of this study was to obtain injectable hydrogel materials based on chitosan and evaluate the effect of different BGs on the crosslinking process and physicochemical, biological and rheological properties of hydrogels. All materials were incubated in SBF solution in order to assess their degradation, while the ICP-OES analysis was held to evaluate the ion migration from/into the solution. After the materials were freeze-dried their microstructure, porosity and mineralization was assessed by SEM/EDX microscopy and ATR-FTIR spectroscopy. Mineralization process in wet state was evaluated using μCT imaging. Additionally, rheological, self-healing properties and injectability (FIG. 1) were investigated. The preliminary *in-vitro* studies of the biological response were carried out on the Human Umbilical Vein Endothelial Cells (HUVEC).

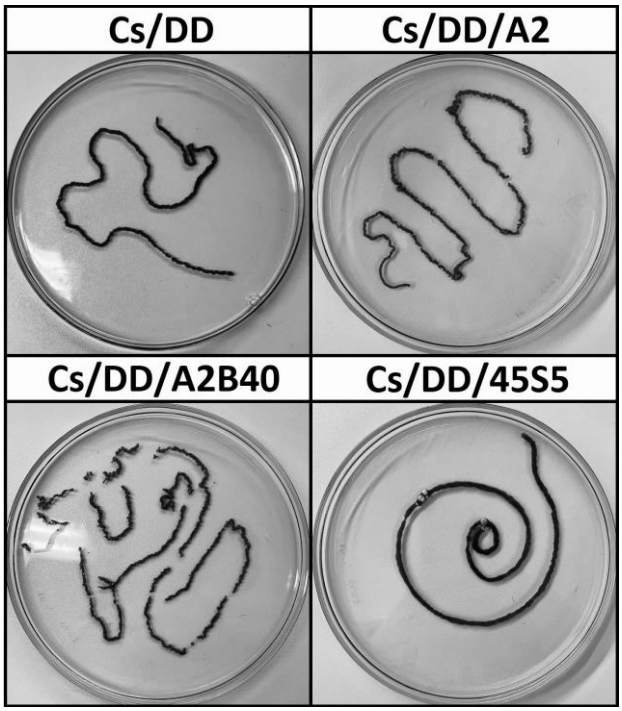

FIG. 1. The injectability of hydrogels into a SBF solution.

Results and Discussion

The results have shown that BGs significantly improve stiffness, compressive strength, and viscoelastic characteristics of hydrogels, as well as greatly reduce crosslinking time. Hydrogels modified with BGs showed good injectability and self-healing properties. Furthermore, no cytotoxic effect of hydrogels was observed in direct contact with HUVECs.

Conclusions

The obtained results clearly confirmed that the developed hydrogels have a great potential to be used as injectable materials for tissue engineering. Furthermore, by using bioactive glasses differ in composition, it is possible to modulate hydrogel properties in a wide range.

Acknowledgments

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MICROSCOPIC STRUCTURE OF HYBRID COMPOSITE BIOMATERIALS BASED ON CARBON NONWOVEN AND POLYSACCHARIDE

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Introduction

Carbon based biomaterials have been used successfully in medicine for many years. However, the constant development of materials engineering creates opportunities to develop newer functional materials for medicine [1,2]. Carbon fibers reveal good biological properties in terms of integration with natural tissues with biocompatibility [3]. Carbon fibres modification, either by chemical or physical methods, increases the scope of their applications. By designing biomedical dedicated composites based on carbon fibers and other biocompatible polymers, such as polysaccharides, it is possible to obtain structures with a specific architecture that will constitute a functional scaffold for cell proliferation processes.
This work presents research on the microscopic structure of composite materials based on carbon nonwovens, which were obtained in two-stage modification. In a first step, on base material, ferromagnetic layer was deposited. In a second step, materials were modified with alginate, resulting in a sandwich composite with an extensive internal structure.

Materials and Methods

Carbon nonwovens obtained from a polyacrylonitrile precursor were used in our studies, on which a ferromagnetic layer was deposited using the PVD method. The description of the carbonization and modification method was presented in our previous work [1]. Carbon nonwovens were modified through the dip-coating process with sodium alginate solution and subsequently crosslinked with $CaCl_2$. The analysis and assessment of the roughness of the composites surface was performed based on the 3D technique using scanning electron microscopy and the Alicona MeX program.
A VEGA3 scanning electron microscope (TESCAN) equipped with an INCA Energy X-ray energy dispersive spectrometer (EDS) (OXFORD Instruments Analytical) was used for SEM/EDS microscopic analysis of the tested materials.

Results and Discussion

Based on the results of the conducted surface roughness analysis it was observed that the obtained structure of composites exhibits not uniform structure on the material surface (FIG. 1). Nevertheless, the obtained histogram of the heights of individual peaks correlates with a Gaussian-like distributions.

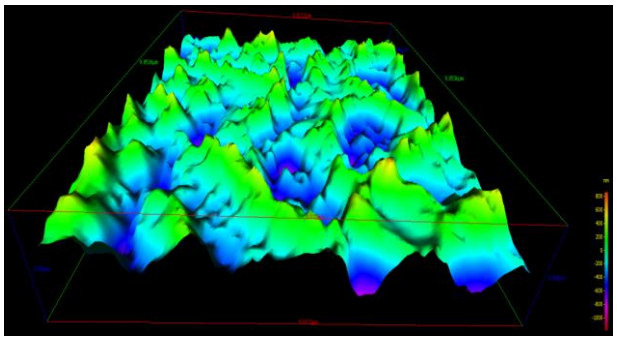

FIG. 1. Visualization of the analyzed sample area using MountainsMaps.

The SEM microscopic analysis indicates that the dip-coating modyfication of carbon nonwovens resulted in an uprising a layered structure with the presence of polysaccharide inside the material (FIG. 2). This structure may have a beneficial effect on the process of cell penetration into the material and increase their lifespan. SEM+EDS analysis showed the presence of characteristic elements originating from carbon fiber, polysaccharide and iron.

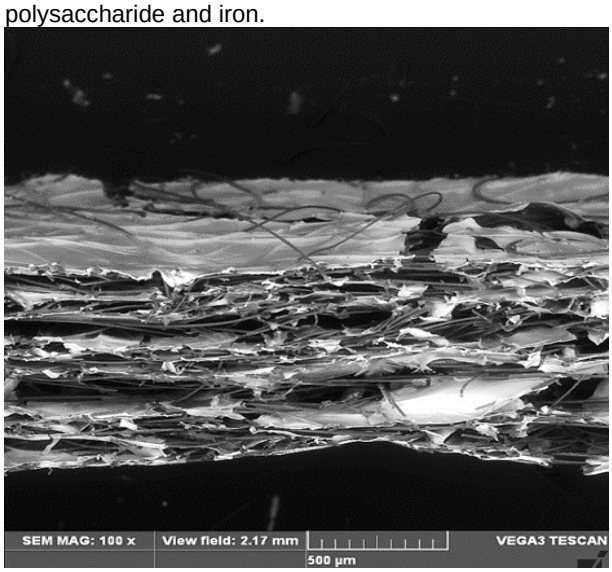

FIG. 2. SEM images of a cross-section of a hybrid carbon material.

Conclusions

The microscopic analysis confirmed the possibility of shaping hybrid materials based on carbon fibers and polysaccharides. At the same time, the presence of ferromagnetic layer on such composite enable veryfication of the biomaterial behavior in the body by using classical imaging methods.

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THE THERMO-PHYSIOLOGICAL COMFORT OF POLYSACCHARIDE NONWOVEN INTENDED FOR WOUND DRESSING MATERIALS

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Introduction

Today's development of wound dressing materials is primarily related to the search for material solutions that will properly protect the wound environment, support the regeneration process, but also monitor the healing process. Nevertheless, appropriate comfort for the patient related to both the dressing replacement process and its long-term wearing is also perceived as important issue. Thus, based on this context, the use of polysaccharide fibers, which exhibit a beneficial effect on the wound healing process as well as are used in many dressing solutions, may be of great importance [1]. Moreover, the appropriate design of the wound dressing ensures comfort of use for the patient.
The aim of the research was to analyze the thermophysiological comfort of selected nonwoven structures based on two types of fibers, i.e. calcium alginate fibers and chitosan fibers.

Materials and Methods

The Alambeta device was used to determine the thermophysiological comfort of nonwovens. This test involves measuring the amount of heat flowing through a sample placed between two plates - the upper one, heated to a temperature of approximately 32°C, and the lower one at 22°C. Additionally, Permetest device was used, in order to mesure the water vapor permeability and thermal resistance of samples.

Results and Discussion

Thermo-physiological properties study results indicate that nonwoven made of 100% calcium alginate has the least favorable parameters compared to chitosan based nonwoven. Nonwoven made of 100% calcium alginate is characterized by the highest thickness h, the highest thermal resistance Rct, water vapor resistance Ret and the lowest thermal conductivity λ (TABLE 1, FIG. 1), which may cause unfavorable thermophysiological comfort in direct contact with human skin. Therefore, in order to increase patient comfort during such wound dressing wearing, it is important to use fiber mixtures with different thermo-physiological parameters.

Conclusions

The conducted thermophysiological comfort tests demostrated that while simultaneously similar surface mass of nonwoven as well as processing technology, the raw material composition has a significant impact on the obtained thermophysiological comfort parameters.
Our studies will constitute the basis for further research on various raw material systems based on polysaccharide fibers.

TABLE 1. Thermophysiological comfort of nonwovens - Permetest studies.

	P %	R_{et} m^2PaW^{-1}	R_{ct} m^2KW^{-1}
Alginate 100	41,98±3,10	8,79±0,58	0,0911±0,0036
Chitosan 100	43,19±1,89	5,99±0,46	0,0598±0,0048
Alginate 50 Chitosan 50	48,88±7,74	7,34±0,28	0,0692±0,0023

R_{et} - water vapor resistance [m^2PaW^{-1}];
R_{ct} - thermal resistance [m^2KW^{-1}];
P - relative water vapor permeability [%]

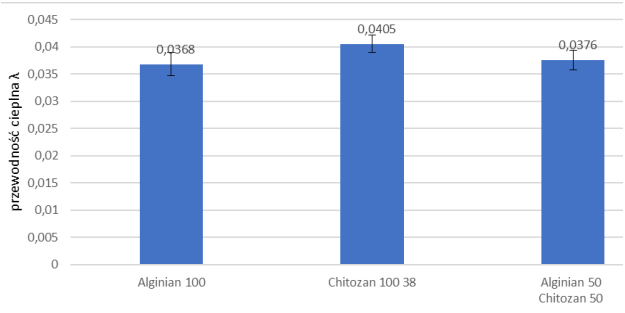

FIG. 1. Thermal conductivity results for polysaccharide nonwovens.

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URSOLIC ACID AS AN ENHANCER OF CHITOSAN FIBRES' ANTIBACTERIAL PROPERTIES

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Abstract

Over the years, chitosan has been the subject of numerous studies and has gained significant popularity as a biomaterial due to its various characteristics, including biocompatibility, biodegradability, and bioactivity. In this work, chitosan fibres' surface was modified with ursolic acid to improve their antibacterial properties by wet impregnation method. Five specimens of chitosan fibres were immersed in ursolic acid (UA) solution for varying immersion times of 1, 2, 4, 6, and 8 hours. Characterization was carried out by means of FTIR, SEM, UV-Vis spectroscopy; the results indicated an ongoing chemical reaction between chitosan and ursolic acid resulting in changes to the chemical structure. After 2 hours, the absorbance ratio remained constant; suggesting that the reaction had reached completion and the chemical structure of the sample remained stable. Antibacterial tests were performed on the resulting chitosan fibres against two bacterial strains. The fibres without ursolic acid did not exhibit any noteworthy antibacterial activity against either strain. However, the chitosan fibres modified with ursolic acid showed significant and almost strong antibacterial activity against the Gram-positive strain, S. aureus. These results suggest that chitosan fibres modified with ursolic acid could have potential applications as antibacterial materials, particularly against Gram-positive bacteria.

Keywords

Chitosan; Fibres; Ursolic acid; antibacterial; Chitosan Fibres

3D BIOPRINTER FOR COMBINED PRINTOUTS MADE OF HYDROGEL BIOINK AND HIGH MELTING TEMPERATURE BIO-THERMOPLASTICS

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Introduction

Natural tissues, as for example blood vessel walls, are built of multiple cell layers, where each layer provides different properties for the tissue [1]. Unfortunately, 3D printing technologies used for medical applications are mostly single or multimaterial process, where materials have very similar characteristics [2-3]. In the worked-out construction of the 3D bioprinter it is possible to join different types of materials (hydrogel bioinks and high melting temperature bio-thermoplastics) to produce tube-like scaffolds in a single process. With possibility to sterilize the working space thus avoiding contamination of the printed material it can provide huge advantage over commercial bioprinters.

Materials and Methods

To maintain the optimal living conditions for the cells the bioink temperature, crosslinking speed and chamber temperature are fully customizable, in the way that high melting temperature do not decrease the cells' viability. Using this multimaterial bioprinter the mechanical properties of the printouts and their accuracy can be improved while the maximal survival rate of cells can be maintained as well. The more, this construction let to join thermoplastics and bioinks layers in single printout during one printing process.

The device has two working spaces, the upper one - for printing with hydrogel materials / bioinks and the lower one - for printing with high melting temperature thermoplastic materials (FIG. 1).

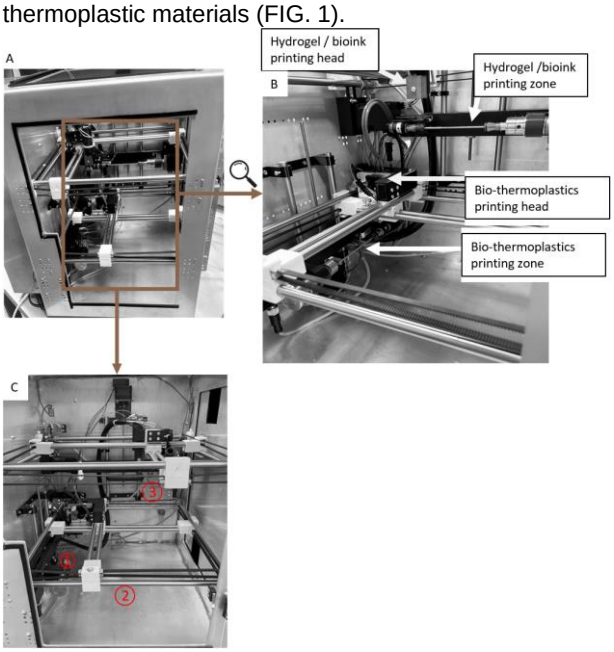

FIG. 1. The picture of a custom designed 3D bioprinter with designated heads and corresponding printing zones for different material types (A, B) and places where the swabs were taken (signed as 1, 2, 3) (C).

In the upper working space of the device, two heads are used to feed the hydrogel material. In the lower working space of the device, a head with direct material feeding was used for thermoplastic filaments. In this way, the length of the filament sections needed to print a single element was reduced to a minimum. Each workspace has a separate and independent control system. The entire chamber of the device has a temperature stabilization provided by the special module to enable thermal cross-linking of gelatine-based materials and to remove process heat appearing during printing with thermoplastic material. What is more, to remove process heat, water cooled extruder was used for thermoplastic material. The chamber of the entire device is sterilized by a set of LED UV C diodes. Their operation is coupled with the stabilization of the chamber temperature, which additionally allows ozone to evenly reach every space of the device, created as a result of the operation of the diodes. After the sterilization process, swabs were taken from three previously selected places (Fig. 1C) in the chamber interior, which were then placed in the medias to assess the effectiveness of the sterilization process. Three medias have been used to check possible colonisation of the widest spectrum of microorganisms: Bacto Brain-Heart Infusion (BD, USA), Sabouraud's broth (BTL, Poland) and Yeast extract peptone glucose (BTL, Poland). Sterility checks were performed after 24 hours, 48 hours and 72 hours. The test was repeated three times.

Results and Discussion

With the described system multimaterial tube like scaffolds have been manufactured (FIG. 2). System allows to bioprint hydrogel layer thickness from 150µm up to 2mm thank to controlled temperature in the chamber which allows for thermal crosslinking of the material. Thermoplastic layer thickness can vary from 180 to 380µm.

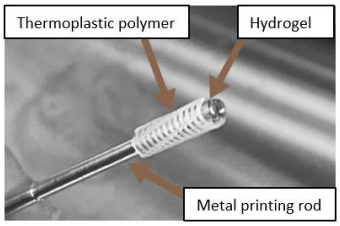

FIG. 2. Presentation of 2 layers printout, where bioink in coupled with thermoplastic polymer.

Sterility checks for all three mediums in all three locations were passed with no microbiological growth observed.

Conclusions

The utilization of the previously mentioned method in creating 3D bioprinted structures from multiple materials could introduce novel opportunities for crafting tubular support frameworks. This approach enables the creation of tube-like scaffolds consisting of a minimum of two layers, wherein the materials employed possess varying melting points without any interaction from the outside of the sterile chamber. These printed constructs could potentially address challenges related to urological disorders like hypospadias.

Acknowledgments

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WATER SORPTION OF DIMETACRYLATE RESIN BLENDS MODIFIED WITH LIQUID RUBBER

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Introduction

Composite materials based on dimethacrylate resins are widely used in dentistry for the restoration of teeth's tissues. They have excellent mechanical and esthetic properties, as well as the ability to bond to the enamel. However, in a humid oral environment, these composites can absorb water based liquids, such as saliva and beverages, which can significantly affect the degradation of the dental composite [1] and leach unreacted monomers [2]. Excessive fluid absorption can have a detrimental effect on the structure and function of the resin [2], as it can reduce the mechanical and physical properties, leading to a shortened life of the dental restoration, and cause the filler to separate from the matrix [3,4]. The purpose of this study was to determine the water sorption characteristics and wetting angle of light-curing resins blend modified with liquid rubber.

Materials and Methods

The material used in the study was a resin mixture with a composition of 20% wt. BisGMA, 30% wt. BisEMA, 30% wt. UDMA and 20% wt. TEGDMA. The Hypro 2000X168LC VTB liquid rubber (Huntsman International LLC, USA) was used as a modifier at 0%, 5%, 10%, 15%, 20% by weight in the resin mixture. Five samples of each material were prepared for water sorption tests. The test method was in accordance with ISO 4049. Samples were dried to a constant weight, then incubated in distilled water at (37±1)°C for 7 days, and daily sample weight measurements were taken. Wetting angle measurement was realized using water of very high quality, the liquid was dispensed at 2µl. The samples were tested 24 hours after polymerization (kept dry). The study was conducted to demonstrate the effect of liquid rubber modification on the wetting angle values.

Results and Discussion

Results of the mass change measurements (in percent) are shown in FIG. 1. A dynamic increase in the mass of the samples was observed during the first three days of incubation, after which the growth rate decreased until saturation was reached. The largest changes in mass were observed for samples with 15 and 20 percent liquid rubber content and for the control sample.

Modification with liquid rubber significantly increased the water contact angle (FIG. 2). Almost all samples showed hydrophobicity (Θ > 65). A raise in the value of the contact angle was observed as the amount of liquid rubber increased. The reasons for the increase in hydrophobicity of liquid rubber modified samples were due to changes in surface topography as well as physicochemical factors. BisGMA resin, as the main component of the composite matrix, has hydroxyl groups, while liquid rubber is non-polar, which favors an increase in the wetting angle.

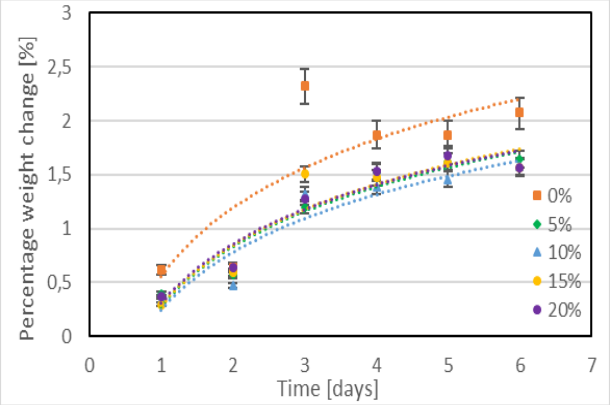

FIG. 1. Percentage weight change.

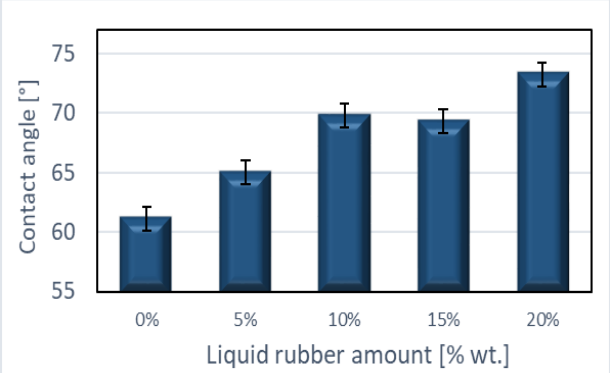

FIG. 2. Results of the contact angle measurements.

Conclusions

Modification of the resins with liquid rubber did not significantly change the water sorption process, however, smaller differences in weight change of the samples were obtained with respect to the control sample. In the case of wetting angle studies, liquid rubber modification significantly increased the water contact angle.

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