ENGINEERING OF
BIOMATERIALS
INŻYNIERIA BIOMATERIAŁÓW
JOURNAL OF POLISH SOCIETY FOR BIOMATERIALS AND FACULTY OF MATERIALS SCIENCE AND CERAMICS AGH-UST
CZASOPISMO POLSKIEGO STOWARZYSZENIA BIOMATERIAŁÓW I WYDZIAŁU INŻYNIERII MATERIAŁOWEJ I CERAMIKI AGH

Number 163
Special Issue
Numer 163
Numer specjalny
Volume XXIV
Rok XXIV

DECEMBER 2021
GRUDZIEŃ 2021

ISSN 1429-7248

PUBLISHER:
WYDAWCA:

Polish Society for Biomaterials in Krakow
Polskie Stowarzyszenie Biomateriałów w Krakowie

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Issue: 250 copies
Nakład: 250 egz.

Scientific Publishing House AKAPIT
Wydawnictwo Naukowe AKAPIT
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30th Biomaterials in Medicine and Veterinary Medicine
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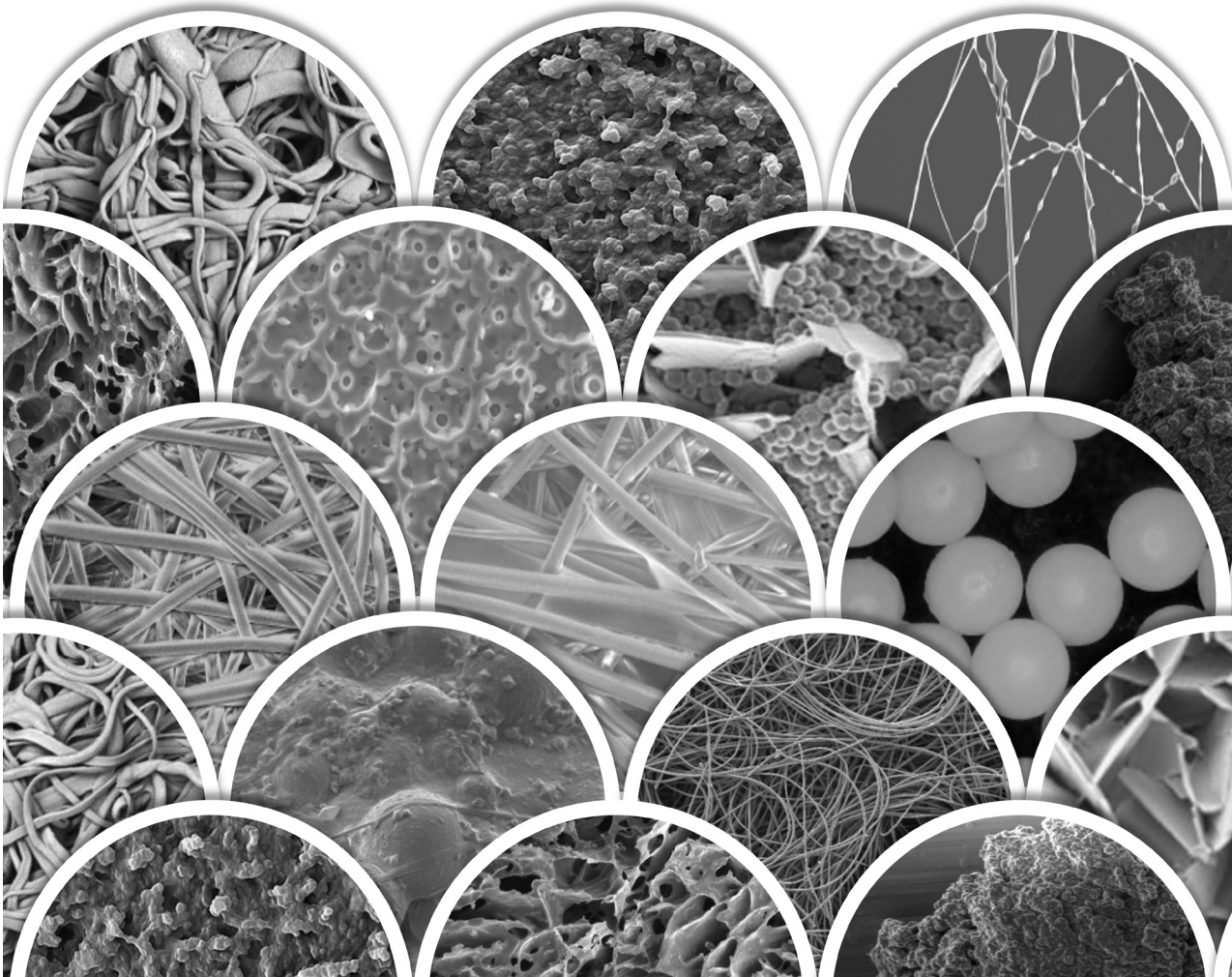

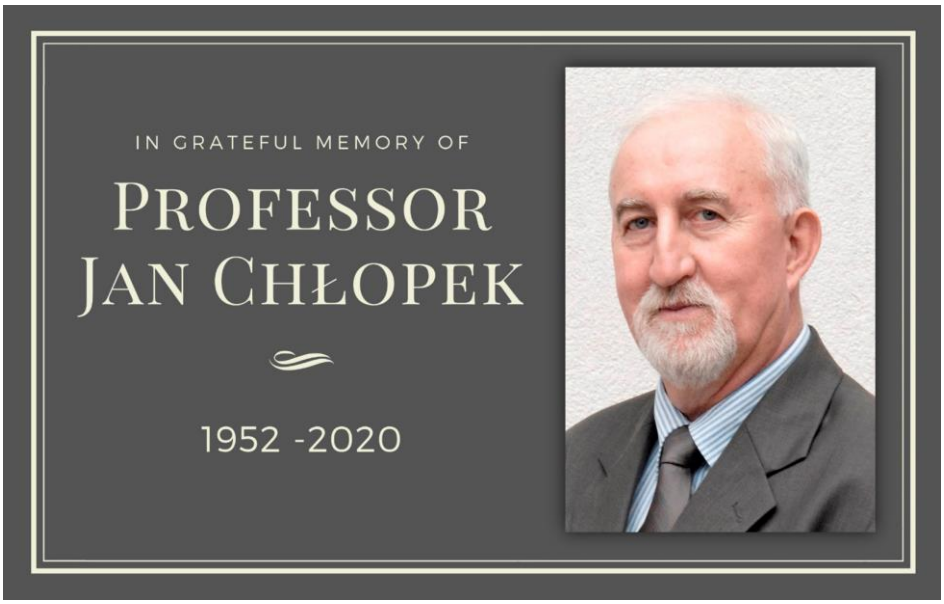

Dear Colleagues and Friends,

This year's conference of the Polish Society for Biomaterials is dedicated to the memory of Professor Jan Chłopek - Chairmen of the 'Biomaterials in Medicine and Veterinary Medicine' annual conferences. We lost our conference Mentor but not the spirit - his memory will be with us while we reminisce the lessons he taught us and the laugh he gave us. It will be cherished throughout the whole conference.

Professor Jan Chłopek passed away on Saturday, November 14, 2020. He was former President of the Polish Society for Biomaterials (PSB), an academic lecturer, a scientist, but above all a wonderful human being, a leader and an extraordinary mentor of students and younger scientists.

For almost 50 years, Professor Jan Chłopek was associated with the Faculty of Materials Science and Ceramics at the AGH University of Science and Technology – first as a student and then as a faculty member. He graduated in materials science in 1976, received his PhD degree in technical sciences in 1983, habilitation in 1998, and finally became a full professor in 2005.

Prof. Jan Chłopek was Dean of the Faculty of Materials Science and Ceramics (2005-2012), Deputy Dean for International Cooperation (2002-2005), Head of the Department of Biomaterials and Composites (2012-2020). He was a founder member of the Polish Society for Biomaterials, where he served as Vice-President (1999-2006, 2010-2013), President (2014-2016) and Council Member (2017-2020). From 2007, he was Editor-in-Chief of the „Engineering of Biomaterials" journal. He was Chairman of the „Biomaterials in Medicine and Veterinary Medicine" conferences, which has been organized annually since 1991. Having gained international recognition as a biomaterial scientist, Professor Chłopek chaired organizing committees of such important scientific meetings as the 27th European Conference on Biomaterials (ESB 2015) and International Conference on Biomedical Polymers and Polymeric Biomaterials (ISBPPB 2018). Prof. Jan Chłopek managed a nationwide team which developed teaching standards for "Biomedical Engineering" in Poland. He was a co-founder of the Multidisciplinary School of Biomedical Engineering at AGH. He received many awards and distinctions for his scientific and teaching activities. As an accomplished figure in the field of biomaterials science and engineering, Professor Chłopek received the accolade of „Fellow, Biomaterials Science and Engineering" (FBSE) from the International Union of Societies for Biomaterials Science and Engineering (IUSBSE).

He was an outstanding scientist working on biomaterials engineering and composite technology. He was the author of over 350 publications, including 7 books/book chapters and 25 patents/patent applications. He was a supervisor of 9 PhD dissertations and over 200 Bachelor and Master students' theses.

The sudden death of Professor Chłopek has left us all in deep grief. He will be remembered as a scientist, mentor, husband, father, grandfather, football fan, player, and coach. We will always cherish his kindness, sense of humor, positive attitude, and faith in people.

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STUDIA PODYPLOMOWE
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2021/2022

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Sylwetka absolwenta:
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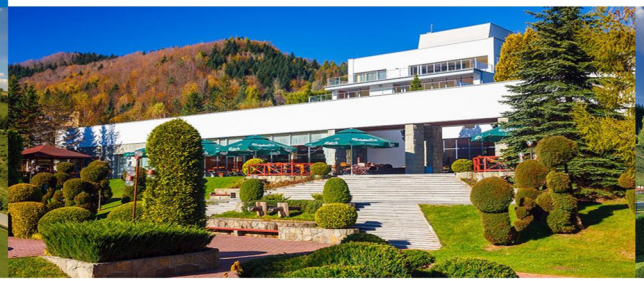

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BIOMATERIALS IN REGENERATIVE MEDICINE: VIEW OF THE PAST & VISION FOR THE FUTURE

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Abstract

In the past two decades, the fields of Tissue Engineering (TE) and Regenerative Medicine (RegMed) have received important input from advances in stem cell research as well as in the biomaterial sciences, including new developments in composite materials and interactive polymer systems. In the latter, for example, biodegradable scaffolds and hydrogels can mimic essential characteristics of the extracellular matrix (ECM), which is the microenvironment of cells in their natural state in situ. Being able to simulate such cell-cell and cell-matrix interactions in vitro is important not only for testing new biomaterials, but also for understanding regenerative mechanisms after implantation. However, this is far from a trivial challenge, although it can be usefully assisted by employing co-culture models in three dimensions. The situation becomes even more complex, when novel biomaterials and strategies for regeneration are investigated in vivo. Testing in animals introduces namely a complexity which makes mechanistic interpretation of observations exceedingly difficult, if not impossible. Moreover, in the past the accepted norms in testing have generally involved, for example, implantation in healthy animals, although in reality most patients receive a biomaterial for a disease state. Thus, for in vivo models there is an acute need to develop relevant models of disease. Future developments must also address the challenges of understanding the effects of, for example, ageing, multi-morbidity and medication on tissue reactions at the implant interface. Such multifactorial considerations play a special role in the case of cancer patients.

In the future, biomaterials and TE & RegMed will be increasingly influenced by the broadening interface with biotechnology. The latter is so vast that it is difficult to put its elements into a single presentation slide which an audience could read without binoculars and a prolonged time slot! However, the COVID-19 pandemic has focussed attention on the power of mRNA technology in modulating the body's immune system. It remains to be established how this technology could be adapted to control unwanted reactions at specific sites, for example, at a tissue-biomaterial interface. Returning to biotechnology as a driver of future progress, it seems highly likely that both major scientific branches of biomaterials, namely the materials sciences and the life sciences, will receive transforming impulses from advances in biotechnology. Fields such as artificial intelligence, green technology, robotics and nanotechnology underline just how diverse biotechnology is. In addition, this diversity stresses the essential role of interdisciplinarity and its implications for university teaching for future generations of materials and life scientists.

FACTORS GOVERNING THE DIFFERENTIATION OF STEM CELLS IN TISSUE ENGINEERING - A REVIEW

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Introduction

Tissue engineering is an interdisciplinary field which applies the principles of biology and engineering to the development of functional substitutes for damaged tissue [1]. For constructing these substitutes, two basic components are needed, namely a biomaterial component, simulating the extracellular matrix of anchorage-dependent cells, and a cell component, which adhere, migrate, proliferate and differentiate on the biomaterial and exert desired functions. Recently, stem cells emerged as a promising cell component with several advantages over the differentiated cells, such as a high proliferation capacity, lower senescence, paracrine production of various growth and immunomodulatory factors and ability to be differentiated into various cell types (for a review, see [2]).

Types of Stem Cells

Stem cells can be divided into four main groups, namely embryonic stem cells, foetal stem cells, adult stem cells, and induced pluripotent stem cells (iPSCs). Embryonic stem cells (ESCs) isolated from blastocyst are pluripotent, i.e. able to differentiate into all cell types except placental cells, but their use in humans is restricted by ethical and legal issues. Stem cells with markers of pluripotency can be obtained from extrafoetal tissues, i.e. from placenta, amnion, chorion or umbilical cord. Among adult stem cells, bone marrow mesenchymal stem cells (bmMSCs) and adipose tissue-derived stem cells (ADSCs) became the most popular due to their relatively good accessibility and availability in relatively high quantities. These cells are only multipotent, i.e. able to differentiate to a limited number of cell types, but they can be routinely used in autologous form. The iPSCs, created by genetic reprogramming of differentiated somatic cells, are associated with a high risk of tumorigenicity due to the use of viral vectors (for a review, see [2]). From this point of view, the adult stem cells are the most promising stem cell type for tissue engineering.

Factors Controlling the Differentiation of Stem Cells

Stem cells can be differentiated into desired cell types by biochemical and mechanical signals. During tissue engineering, which is carried out mainly under *in vitro* conditions, the main source of biochemical signals is cell culture medium. Mechanical stimulation is provided by dynamic cell culture systems. Cell adhesion substrate can be a source of both biochemical and mechanical signals.

Composition of the cell culture medium. Each direction of cell differentiation requires the presence of specific growth factors and other biomolecules in the culture medium. For example, differentiation towards vascular smooth muscle cells (VSMCs) occurs when the medium is supplemented with transforming growth factor-beta 1 (TGF-β1) and bone morphogenetic protein-4 (BMP-4) [3], and differentiation towards endothelial cells (ECs) requires the presence of vascular endothelial growth factor (VEGF) [2]. Osteogenic medium contains β-glycerophosphate, dexamethasone, and vitamins C and D3 [3,4], and the medium for differentiation towards keratinocytes is supplemented with epidermal growth factor or keratinocyte growth factor [2].

Mechanical stimulation of cells. Each direction of stem cell differentiation also requires specific type of mechanical stimulation. For example, cyclic strain is needed for differentiation towards VSMCs, while laminar shear stress promotes the differentiation towards ECs. Osteogenic cell differentiation is stimulated by vibrational stress, and the differentiation towards keratinocytes by pressure or uniaxial strain stress. Mechanical stimulation can be, at least partly, substituted by electrical or magnetic stimulation [2].

Properties of the cell cultivation substrate. The differentiation of stem cells can also be influenced by the composition, architecture, physicochemical and mechanical properties of the cultivation substrate, such as its wettability, functionalization with various biologically active substances, two- or three-dimensional architecture, and particularly stiffness. For example, very soft substrates, having mechanical characteristics similar to those of brain tissue, direct the differentiation of stem cells towards neurons. On stiffer substrates, mimicking the muscle tissue, the stem cells became myogenic, and on the stiffest matrices, the stem cells differentiate towards osteoblasts (for a review, see [2]).

Differentiation of stem cells into difficult-to-reach cell types

The differentiation of adult stem cells, particularly of those of mesenchymal origin (bmMCS, ADSCs), towards more specialized cells, such as endothelial cells or keratinocytes, is considered difficult and is scarcely achievable by the factors mentioned above. The reason is the polarization (i.e. functional specialization) of the basal and apical cytoplasmic membrane or the need of transdiferentiation from mesodermal to ectodermal cells. In this case, the cell reprogramming becomes necessary. Modern approaches offer this reprogramming without genetic manipulation, namely by using synthetic messenger RNAs (mRNAs) or self-replicating RNA, which encode markers characteristic for pluripotent stem cells, such as Oct4, Klf4, c-Myc and Sox2, and do not integrate into the host genome [5].

Conclusions

Stem cells are promising cell component for advanced tissue engineering and can be differentiated in a variety of cell types using biochemical and mechanical signals arising from the appropriate composition of cell culture media, cell adhesion substrate and mechanical stimulation. When the differentiation by physiological signals is difficult and incomplete, generation of pluripotent cells by non-genome integrating methods will facilitate possible clinical application of these cells.

Acknowledgments

The study was supported by the Grant Agency of the Czech Republic (grant No. 21-06065S) and the Ministry of Health of the Czech Rep. (grant No. NV18-02-00422).

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3D BIOPRINTING - DEDICATED BIOMATERIALS AND DEVICES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 9]

Introduction

3D bioprinting became a promising approach for fabrication the complex biological constructs in the field of tissue engineering and regenerative medicine. It aims to overcome limitations of conventional tissue engineering methods by precise and controlled layer-by-layer assembly of biomaterials in a desired 3D pattern [1].

A traditional method in fabricating 3D tissue scaffolds involves seeding cells into a scaffold that provides structural and functional support to facilitate tissue regeneration. However, it is not applicable for tissues and organs with complex structure, as it does not provide a uniform cell distribution, has low cell density, slow vascularization, and limitation in the diffusion of nutrients and by-products. To address these issues, three-dimensional (3D) bioprinting was utilized and explored for the fabrication of tissues and organs using biomaterials, specific cells, and bioactive growth factors to promote tissue regeneration and effectively restore its functions.

A direct bioprinting allows the introduction of biological material in the entire volume of the printed object. Research work on cells, tissues and organs printing is aimed at fulfilling demands of organ shortage, cell patterning for better tissue fabrication, and building better disease models.

An indirect bioprinting is the production of structures that do not contain biological material, but can fulfill their specified function (supporting, protective, scaffolding for overgrown tissues, etc.), remaining biocompatible with the cells, tissues and fluids with which they are in contact. Both of these bioprinting branches have a revolutionary impact on biomedical engineering and medicine.

Materials and Methods

The two important factors that determine an effective 3D bioprinting process are the bioink/biomaterial and the bioprinter.

Biomaterials use for 3D printing process can represent various groups: polymers, ceramics, hydrogels, and metals. Different parameters should be considered in choosing materials for bioprinting [5]. Ideal material should be biocompatible, has appropriate mechanical and rheological properties to withstand bioprinting process and degradation [6].

The most commonly used 3D printing technologies for biomedical applications can be broadly categorized as either extrusion [2], particle fusion-based, droplet [3], or laser-based [4]. Each of these categories contains subgroups that use slight mechanical or chemical variations on each technique, which affect the material properties required for successful design and printing of the ink material. Each of these techniques has dedicated printers or variations of the existing ones.

The latest literature reports present countless studies on the possibilities of different bioprinting modalities. The selection of bioinks for each of them usually varies based on the ink's rheology, viscosity, crosslinking chemistry, and biocompatibility. Significant advancements have been made to integrate secondary techniques accompanying the modalities of bioprinting [7].

Results and Discussion

Two main aspects related to 3D bioprinting will be discussed: 1) materials that can be used as a bioink (for both indirect and direct printing) – what are the limitations and biggest challenges for them currently and 2) printing devices that can meet the requirements related to the increasing complexity of printed objects and their decreasing sizes determining the need to achieve very high printing accuracy.

Hydrogel materials are very good materials for the bioink, although there are still many parameters that pose a challenge in terms of obtaining their assumed and repeatable features. The possibility of introducing additional components into them allows for targeting their properties in terms of a given application in a given area of medicine. The bioactivity and properties of alginate-based hydrogels are deteriorated not only by their composition, but also by the order of mixing, the solvent used, type, cross-linking time and concentration of the cross-linking solution. On the other hand, the properties of hydrogels mean that they create protective conditions for cells during 3D printing, thanks to which it is possible to maintain their high survival rate.

In the field of thermoplastic polymers, a very desirable property is their controlled degradation, including degradation in a very short time corresponding to the tissue healing rate. Such possibilities can be obtained by combining various polymers, which creates a real challenge to create a suitable filament from them, in order to then be able to verify printability, accuracy and properties after thermal treatment, which is the 3D printing process itself.

Conclusions

Printability of a biomaterial is strictly determined by the printing technique. Although it is possible to print the same material using multiple printing techniques, the form and composition of the printable material, varies significantly. The applications of 3D bioprinting are not limited to organ printing. It also holds great promise in less explored avenues, such as using scaffolds for drug delivery, studying disease mechanisms, or creating personalized medicines. Currently, a limited number of bioinks exist which are both bioprintable and which accurately represent the tissue architecture needed to restore organ function post-printing.

Acknowledgments

Research conducted within the project MATURO 3D (TECHMATSTRATEG2/407770/2/NCBR/2020) financed by The National Centre for Research and Development, Poland.

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CURE FOR TYPE 1 DIABETES WITH BIOTECHNOLOGY – HOW FAR WE ARE FROM CLINICAL APPLICATION?

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[ENGINEERING OF BIOMATERIALS 163 (2021) 10]

Introduction
Type 1 diabetes (T1D) is an autoimmune disease, which affects the huge number of patients. Islet or pancreatic transplantation is a method of treating patients with diabetes mellitus. The limitation of these methods is the lack of organs for transplantation. The problem may be solved by 3D-bioprinting technology. 3D-bioprinting using living cells is the latest technique in the field of biomedical engineering. In this study, there are the results of bioprinted bionic pancreas tissue and the first studies on mouse and pig model.

Materials and Methods
Research was carried out on 60 mice (SCID) and 24 pigs. The mice were divided into 3 groups: control; IsletTx in which porcine pancreatic islets were transplanted under the renal capsule; 3D-bioprint in which bioink petals consisted of bioink A and porcine islets. The bioprinted petals were transplanted into the dorsal part of the muscles under the skin in mice. Daily glucose measurement was performed and the level of C-peptide was tested every 7-days.
The pigs were divided into 3 groups: control, diabetic group after pancreatectomy (T1D); induced diabetes group (after pancreatectomy), with transplanted in 7 days 3D-printed bionic petals (TX). The bionic petals were implanted under the peritoneum. The animals were measured daily with blood glucose levels (from 5-20 measurements per day). The pigs also showed a significantly lower insulin requirement after petals implantation.

Results and Discussion
The results obtained in mice initially showed no differences in the concentration of peptide-C and glucose between groups. However, as early as 7-days after transplantation, both parameters analyzed in the fasting state were significantly lower in the IsletsTx and 3Dbioprinted groups compared to the control group. On day 14, decreased values of C-peptide and glucose were observed only in the group with petals transplants.
The results of the observations in pigs showed a decrease in the mean blood glucose level 48 hours after the transplantation of the petals. Mean glucose levels were two times lower, compared to the period before petals transplantation. In addition, TX pigs required lower doses of insulin after petals implantation.

Conclusions
Transplantation of bionic petals in mice and pigs resulted in a decrease in mean glucose levels. The mice showed a reduced concentration of their own C-peptide, which can indicate relief in mice's owns islets function. None of the animals died due to postoperative complications or the lack of biocompatibility with the bionic structure. Positive effect of transplantation was maintained throughout the experiment, which proves the optimal selection of the composition of the bioink and bioprinting parameters.

Acknowledgments
Project „3D – BIOPRINTING OF SCAFFOLDS USING PANCREATIC ISLETS OR INSULIN PRODUCING CELLS IN ORDER TO CREATE BIONIC PANCREAS" funded by National Center for Research and Development in the framework of the programme „Prevention and Treatment of Civilization Diseases" STRATEGMED. Timeframe: 01.01.2017 – 30.06.2021.
Accomplished by the Bionic Consortium - Foundation of Research and Science Development, Medical University of Warsaw, Warsaw University of Technology, Nencki Institute, MediSpace sp. Z o.o.

3D BIOPRINTED HUMAN TISSUE MODELS FOR PHARMACEUTICAL AND COSMETIC PRODUCT TESTING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 11]

Introduction

Founded in 2016, CELLINK is the leading bioconvergence company in the world, providing technologies, products and services in bioprinting, multi-omics, cell line development and diagnostics. The company develops innovative technologies for 3D cell culture, high-throughput drug screening and printing human tissue and organ models for the medical, pharmaceutical, and cosmetic industries.
This work is essential for developing relevant *in vitro* models since 2D models in drug screening can lead to both false positive and false negatives, extending the time and resources needed for the drug to reach the patients [1]. There is also a great need for better *in vitro* skin models for cosmetic product testing to limit animal trials [2]. The aim is to fabricate many different types of functional tissues using 3D bioprinting as more reliable models for pharmaceutical and cosmetic product testing.

Materials and Methods

3D bioprinted constructs are fabricated layer by layer using BIO X bioprinter while living cells are embedded in biomaterials. CELLINK's bioinks are groundbreaking biomaterial solutions that enable researchers to culture human cells into functional tissue constructs. These bioinks provide an environment similar to native human tissue that cells can thrive in due to adhesion contacts, as well as the ability to be manipulated and remodeled, and direct differentiation and organization. After bioprinting, the constructs are cultured using standard culture conditions for 1-4 weeks for maturation.

Results and Discussion

A broad variety of tissue models can be printed using the BIO X bioprinter for pharmaceutical and cosmetic product testing such as cancer tumor models, heart, liver and skin.
Cells have the ability to remodel their environment and migrate forming spheroids within the bioprinted construct which can be used for cancer drug screening (FIG. 1).

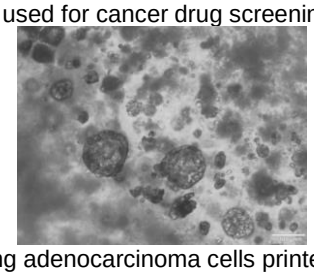

FIG. 1. Lung adenocarcinoma cells printed in GelMA bioink have migrated and formed spheroids after 16 days.

Human iPSC-derived cardiac aggregates were printed in CELLINK LAMININK 521 and after three weeks of culture, the bioprinted cardiac tissue model demonstrated intracellular mobilization of calcium (FIG. 2). These models can be utilized to investigate cardiomyocyte maturation, drug screening, identifying drug targets, and cardiac regeneration.

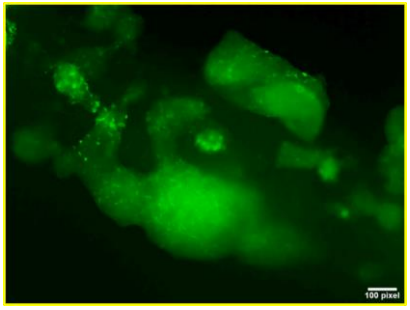

FIG. 2. Cardiac aggregates in CELLINK LAMININK 521 during a fluorescent calcium indicator assay.

Liver tissue models are being used for drug development and to study absorption, distribution, metabolism and excretion of drugs. For sensitive primary liver cells the bioink HEP X, which contain decellularized liver extracellular matrix, will give the cells the best prerequisites for functional tissues (FIG. 3).

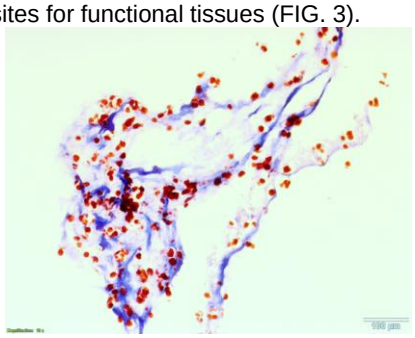

FIG. 3. Trichrome staining of constructs with primary hepatocytes in liver extracellular matrix bioink cultured for two weeks. (Cells-red; ECM-blue).

The different compartments of the skin can easily be layered with different cell types and bioinks using 3D bioprinting (FIG. 4). Skin tissue models can be used for drug and compound treatment to study the cellular response.

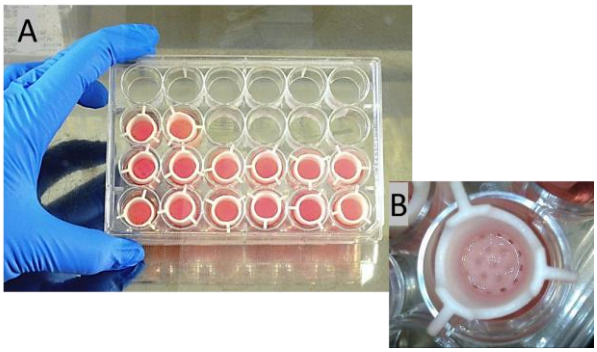

FIG. 4. Bioprinted skin constructs in transwell inserts in A) 24-well plate B) viewed from above.

Conclusions

Various types of 3D bioprinted human tissue models can be used as reliable models to improve the current pharmaceutical and cosmetic product testing.

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HUMAN SPONGY BONE EXPLANT – A USEFUL *EX VIVO* MODEL FOR IMPLANT OSSEOINTEGRATION TESTING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 12]

Introduction

Permanent bone implants should exhibit some crucial features, enabling their good osseointegration (formation of a direct connection with the host bone tissue) after their implantation into the organism [1]. It may be assumed that implant that shows good bioactivity and osteoconductivity (ability to promote osteoblast adhesion, proliferation, and osteogenic differentiation) under *in vitro* conditions, should also provide good osseointegration *in vivo*. Nevertheless, according to available literature, the only method to confirm implant osseointegration is to perform *in vivo* animal tests. On the other hand, the use of animal models at preliminary stage is against the principles of the '3Rs', aiming to Replace, Reduce and Refine the use of animals wherever possible. The aim of the study was to determine the osseointegration process using *ex vivo* explant model. For this purpose, human spongy bone explant was drilled and filled with chitosan/curdlan/HA biomaterial followed by its long-term culture under *in vitro* conditions. In this research, chitosan/curdlan/HA biomaterial was used as a model bone implant due to its high biocompatibility and osteoconductivity that was demonstrated under *in vitro* conditions in our previous studies [2,3].

Materials and Methods

Femoral head of the patient undergoing total hip replacement surgery was used in the study after obtaining informed consent and approval of the Bioethics Committee of Medical University of Lublin (no. KE-0254/74/2020, 30 April 2020). The spongy bone was cut into small pieces (approx. 8 mm x 8 mm and 5 mm in height) that were drilled to obtain the 3 mm-diameter defects. The defects were then filled with the chitosan/curdlan/HA biomaterial and the explants were subjected to long-term culture under *in vitro* conditions: 25 days in a complete culture medium followed by 21 days in the complete culture medium supplemented with 50 μg/ml L-ascorbic acid and 10 mM β-glycerophosphate to induce bone extracellular matrix (ECM) synthesis by the osteoblasts [4]. After 46-day culture of the bone explant, its viability was confirmed by fluorescent staining using calcein-AM (green fluorescence of viable cells) and propidium iodide (red fluorescence of nuclei of dead cells). Osseointegration process was determined by evaluation of osteoblast growth between the biomaterial surface and the host bone by SEM and confocal laser scanning microscopy (CLSM). Moreover, newly deposited ECM (collagen, fibronectin) within the bone-biomaterial connection was assessed using immunofluorescence and CLSM observation.

Results and Discussion

This study presents for the first time *ex vivo* determination of osseointegration process using human spongy bone explant that was drilled and filled with the biomaterial followed by its long-term culture under *in vitro* conditions. Performed experiments clearly proved that human bone explant may stay alive for a long period of time (at least for approx. 50 days). Live/Dead staining revealed that surface of the bone explant was covered by viable osteoblasts that outgrew from the tissue. Furthermore, Live/Dead staining showed also viable cells at the bone-biomaterial interface, proving material osseointegration with the bone explant. Formation of the direct connection between the bone explant and biomaterial was also visualized by SEM (FIG. 1) [4].

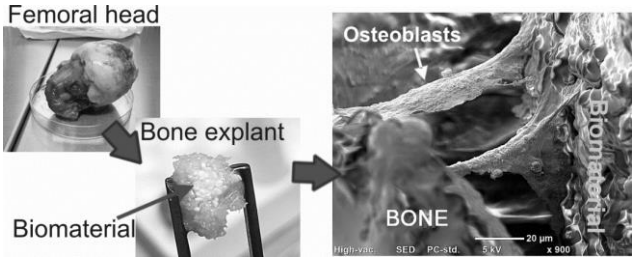

FIG. 1. Schematic representation of the main concept of the study and SEM micrograph presenting the sheets of osteoblasts formed between the bone and biomaterial [4].

Moreover, osteoblasts were demonstrated to have the ability to produce bone ECM (type I collagen, fibronectin) at the bone-implant interface, which was proven by immunofluorescence and CLSM observation (FIG. 2).

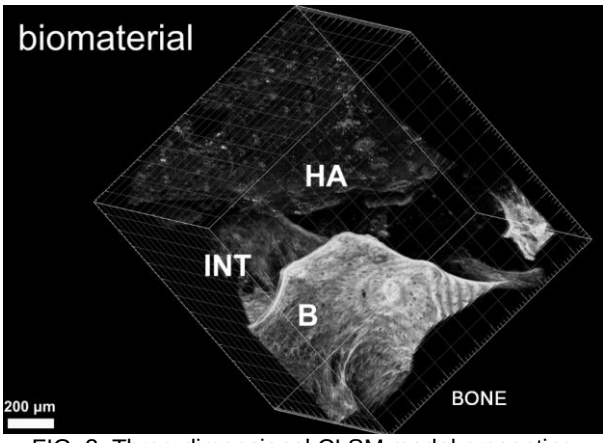

FIG. 2. Three-dimensional CLSM model presenting osseointegration and ECM deposition between the bone tissue and the biomaterial (B – bone tissue, HA – hydroxyapatite granule of the biomaterial, INT – osteoblasts and ECM at bone-biomaterial interface) [4].

Conclusions

Within this study it was demonstrated that *ex vivo* bone explant, which is a heterogeneous tissue containing many different cell types, may serve as an excellent model to test biomaterial osseointegration during preliminary studies, reducing animal tests which is compatible with the principles of '3Rs', aiming to Replace, Reduce and Refine the use of animals wherever possible.

Acknowledgments

The research was supported by the Ministry of Education and Science in Poland within the statutory activity of the Medical University of Lublin (DS3/2021 project).

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THE POLYMERIC EPD - THE INFLUENCE OF PROCESS PARAMETERS AND SOLUTION CHARACTERISTICS ON THE ELECTROPHORETIC MODIFICATION OF FIBROUS CARRIERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 13]

Introduction

The mechanism of electrophoretic deposition (EPD) is a simple and versatile method of surface modification which is used for the preparation of many different materials. It results in specific biological, chemical or mechanical characteristics and is predominantly used for metallic biomaterials or deposition of metals and oxides. Unfortunately, the knowledge of electrodeposition on fibrous carriers is very limited due to its complexity, uneven structure and specific spatial characteristics which can hinder the application of this method in textiles and in general - fibrous structures. In the study, two groups of parameters were investigated – the influence of the EPD process parameters (voltage, time, temperature) and solution characteristics (concentration, conductivity, surface tension) one the kinetics of the electrophoretic deposition of hyaluronate on the spun bonded PLA matrices. The kinetics was measured as mass gain of the substrate with the polymer layer over time.

Materials and Methods

The solutions of the selected concentration of sodium hyaluronate with two molecular weight variants (SH): M_w = 1,8 – 2,0 MDa and M_w = 80 – 130 kDa (Contripo Biotech, Czech Republic) were prepared using distilled water and mechanical stirring for 5 h. The solutions were used after degassing in ambient temperature. The PLA nonwovens were obtained by the spun-bond method and characterised in terms of surface mass, thickness, and air permeability with respective standards (PN-EN). The results are presented in TABLE 1. Surface tension measurements of deposition solutions were performed with a process tensiometer Radian Series 300 (Thermo Scientific, United Kingdom) using Wilhelmy's plate method. The conductivity of deposition solutions was measured with a multifunctionmeter – CX-701 (Elmetron, Poland) and conductivity sensor – EC-60 at T_{ref} = 25°C. The zeta potential was measured with Zetasizer Nano ZS (Malvern Analytical). The electrodeposition was carried out in the dedicated laboratory stand using the voltage range 15 – 60 V, temperature scope 15, 35° and 60°C and time 3-15 min. The samples were dried at room temperature until they reached a constant mass. The effectiveness of the process was measured based on the mass gain [g] over time. The analysis of surface morphology of samples was performed using scanning electron microscopy (FEI NOVA NanoSEM 230) equipped with a field emission electron gun (FEG). The FTIR analysis of the bulk sodium salts, unmodified and modified non-wovens was performed with the FTIR spectrophotometer (Thermo Scientific. Nicolet 6700). The measurement resolution was 4 nm and 64 scans were taken during the measurement.

TABLE 1. The structural characteristic of the spun-bonded PLA nonwoven

Surface mass	Thickness	Air permeability	Fibre diameter
[g/m^2]	[mm]	[l/m^2/s]	[um]
62.2	0.27	480	9.5

Results and Discussion

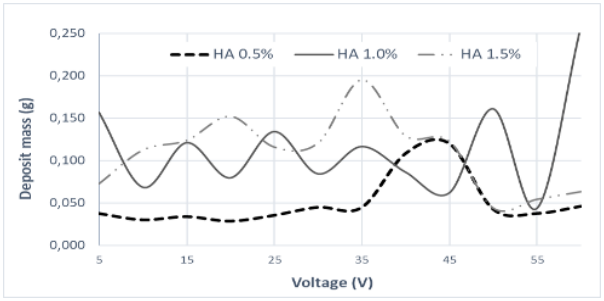

FIG. 1. The influence of polymer concentration and applied EPD voltage on deposit mass, EPD: 35V, 3 min.

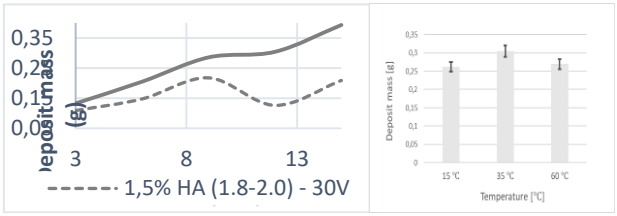

FIG. 2. The influence of voltage, temperature and time on deposit mass.

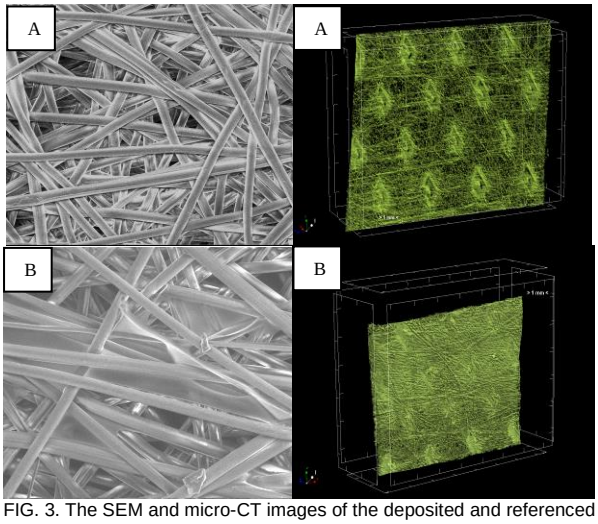

FIG. 3. The SEM and micro-CT images of the deposited and referenced samples. Solution: 1.5% HA, EPD: 35V, 3 min: A-reference, B-deposited.

Conclusions

The effectiveness of the EPD process depends on number of factors. Two main groups of parameters can be pointed – the process parameters and the deposit solution's characterization which play the most important roles. The study resulted in ordering of the knowledge on polymeric EPD on fibrous structures and allowed to indicate the selection of optimal conditions for the deposition of hyaluronic acid on fibrous PLA-based carriers. Based on the examination, the most optimal conditions were selected, i.e. sodium hyaluronate of M_w = 1,8-2,0 MDa, the concentration of 1,5% and process parameter V = 35V, T = 35°C and t = 3-15 min, for the surface modification of spun-bonded nonwovens.

Acknowledgments

The project was financed by National Science Centre in Poland under the contract no UMO-2015/19/N/ST8/02456.

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THE EFFECT OF NANO-HA-BASED BIOMATERIAL ON MACROPHAGE POLARIZATION AND OSTEOGENIC DIFFERENTIATION IN CO-CULTURE SYSTEM *IN VITRO*

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[ENGINEERING OF BIOMATERIALS 163 (2021) 14]

Introduction

Biomaterials for tissue engineering applications should not induce inflammatory reactions after implantation, avoiding implant loosening. Macrophages play a critical role in the regulation of biomaterial-induced inflammatory response. Phenotype 1 macrophages (M1) were described as the pro-inflammatory type, whereas phenotype 2 macrophages (M2) were considered as the anti-inflammatory type [1]. The aim of this study was to evaluate the effect of the novel nanoHA-based bone scaffold (Polish Patent no. 235822) on the macrophage polarization and osteogenic differentiation.

Materials and Methods

The tested biomaterial was composed of chitosan, agarose, and nanohydroxyapatite (nanoHA) and it was prepared in accordance with the method described previously [2]. Macrophages were obtained by the differentiation of human acute monocytic leukaemia cells (THP-1, ATCC) in response to phorbol 12-myristate 13-acetate (PMA) stimulation. THP-1-derived macrophages (M0 phenotype – nonpolarized macrophages) were polarized to M1 and M2 phenotypes by exposure to LPS/INF-γ and IL-4/IL-13, respectively, as shown in FIG. 1. Macrophage characterization was conducted by assessment of levels of proinflammatory (IL-1β, IL-6) and anti-inflammatory (IL-4, Il-10, IL-13, TGF-β1) cytokines using commercially available human-specific ELISAs and by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin.

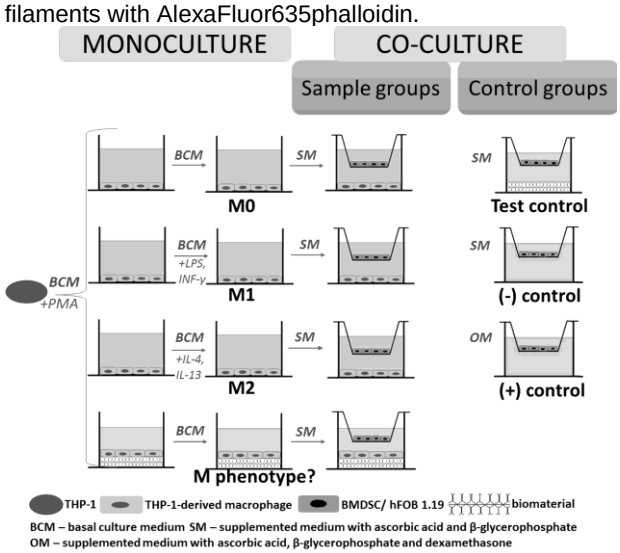

FIG. 1. Scheme showing the main idea of the co-culture experiment [3].

The co-culture experiments were conducted by co-culturing the THP-1-derived macrophages (M0, M1, and M2 macrophages seeded into PS wells and onto the surface of the biomaterial) with human bone marrow-derived stem cells (BMDSC, ATCC) to confirm the paracrine effect of macrophages on osteogenic differentiation, as shown in FIG. 1. On the 6th day of culture, collagen type I (Col I) and bone alkaline phosphatase (bALP) levels were assessed using commercially available human-specific ELISAs. On the 21st day, osteocalcin (OC) and bALP levels in the cell lysates were determined.

Results and Discussion

Comparative analysis of the secretion profile of cytokines and growth factors in monoculture of M0 (nonpolarized macrophages), M1, M2 macrophages and cells cultured on the surface of the developed bone scaffold revealed that macrophages grown on the tested biomaterial released a high level of anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGF-β1), which is typical of the M2 phenotype. Moreover, an assessment of cell morphology confirmed M2 polarization of the macrophages on the surface of the biomaterial (FIG. 2). In turn, evaluation of the level of typical osteogenic markers showed that BMDSC co-cultured with macrophages-seeded biomaterial produced a significantly higher amount of Col I, bALP, and OC than monoculture of BMDSCs grown in the presence of biomaterial (test control). Thus, it was demonstrated that M2 macrophages had a positive effect on the osteogenic differentiation of BMDSCs.

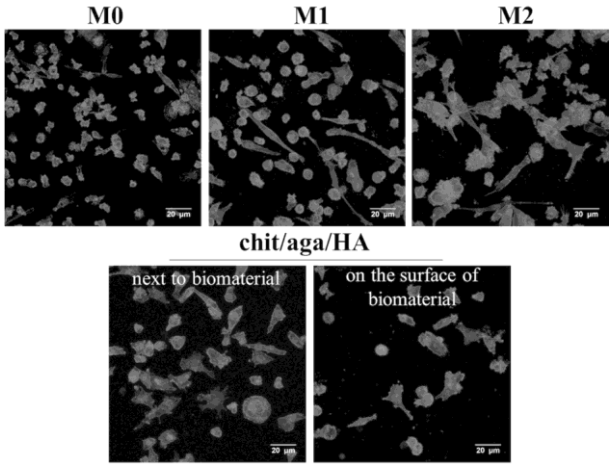

FIG. 2. Macrophages morphology after 3-day culture in the polystyrene wells (M0, M1, and M2 macrophages), on the developed scaffold, and next to the biomaterial [3].

Conclusions

In this study we demonstrated that the novel developed bone scaffold induced M2 polarization. The co-culture of macrophages-seeded nanoHA-based biomaterial with mesenchymal stem cells enhanced their osteogenic ability, approving the immunomodulatory effect of the macrophages on the osteogenic differentiation process. Moreover, we proved that developed biomaterial carries a low risk of inflammatory reactions and thus is a very promising bone scaffold for regenerative medicine applications.

Acknowledgments

Paulina Kazimierczak has received annual support (scholarship – START) from the Foundation for Polish Science (FNP) in 2021 for the most talented young scientists. The study was supported by National Science Centre (NCN) in Poland within OPUS 16 grant no. UMO-2018/31/B/ST8/00945.

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DEVELOPMENT OF A 3D-PRINTED PCL-TiAlNb MATERIAL COMPOSITE WITH ANTI-MICROBIAL ZnO COATING FOR NEXT-GENERATION MAXILLOFACIAL IMPLANTS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 15]

Introduction

Jawbone resection is the final surgical treatment for ~5500 patients in EU27 with maxillofacial benign and malignant tumours. The resulting large bone defects lead to scarred, mangled facial appearance and the loss of mastication and speaking function, requiring aesthetic and functional reconstruction as basis for physical and physiologic rehabilitation. Although autologous vascularized bone from fibular or iliac-crest autografts is current gold standard, the portion of transplantable bone is limited and subsequent high-dose anti-cancer chemo-/radiotherapy often results in tissue necrosis.

Alternatively, a final reconstruction was based in current research activities on patient-specifically manufactured maxillofacial implants without autografts, whereby the neoformation of vascularized bone in such implants occurs within the patient's own body as "bioreactor" as the safest approach in tissue engineering. Compared to the state-of-the-art Ti implants, the metal-polymer hybrid implants were targeted on fulfilling the functional (mastication, speaking, etc.) and aesthetical jawbone reconstruction needs together with strongly improved accuracy (dental interocclusion), mechanical strength, antimicrobial protection, low irritation of surrounding tissue and the possibility for CT imaging in oncological re-checks.

Materials and Methods

The hybrid implants were manufactured by following additive manufacturing techniques:

- selective laser melting (SLM) of V-free TiAlNb alloys and isostatic pressing for the fatigue- and corrosion-resistant metal CORE
- fused filament fabrication (FFF) of polycaprolactane (PCL, PolyMed Inc.) with in vivo biodegradation duration of >1 year for bone neoformation within the biomimetic pore structure (60% open porosity with >0.7 mm pore size) for the bioresorbable polymer scaffold SHELL

The surfaces (including the open porosity) were optimized by ZnO coating deposition (low-temperature thermal atomic layer deposition, ALD) for anti-microbial protection over >12 weeks.

The presented biomaterial characterization was based in vitro on cytotoxicity testing (direct contact and eluates in contact to fibroblasts), genotoxicity testing (MILLIPLEX® MAP 7-plex DNA Damage/Genotoxicity Magnetic Bead) and anti-microbial testing for Staph. aureus & epidermidis, E. coli according to ISO 22196. In vitro testing used the sheep model (3 months implantation in femur bone).

Results and Discussion

The in vitro results showed no cytotoxicity in direct contact as well as to eluates for the tested fibroblasts and all material combinations (uncoated as well as ZnO coated surfaces). The anti-microbial behaviour of the ZnO coated PCL and TiAlNb surfaces revealed a decrease from 1E5 to <1E1 Staph. aureus and E.coli species within 24 h, while bacterial growth occurs on non-coated surfaces. Genotoxicity assay results don't indicate any impact on gene expression.

In vivo tests are currently ongoing and will be finalized till November 2019. Interim results, obtained by microCT, do not show any adverse effect on the in-ingrowth of the specimen into femur bone for all the tested combinations as well as the reference materials (pure Ti). The comparison of in vivo and in vitro results will mainly be based on comparison of histological and microCT findings with the found mechanisms of dissolution of the bioresorbable PCL substrate and ZnO coatings including scanning electron microscopy studies, e.g. structure formation on the surface during dissolution, interface formation between bone and biomaterials.

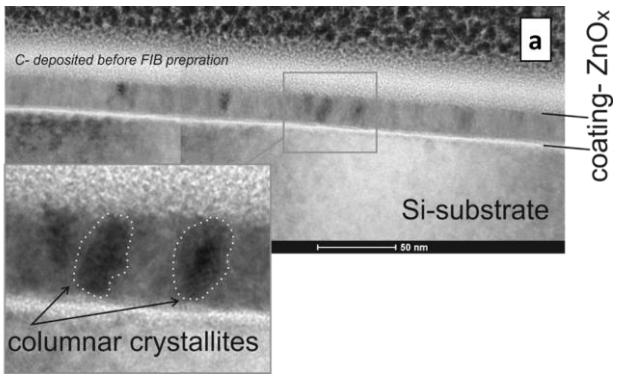

FIG. 1. (a) Transmission electron microscopy image of the ZnO coated surface. (b) Final prototype of the composite implant.

Conclusions

These comprehensive developments will have crucial material, technological & biomedical impact on maxillofacial tumour surgery.

Acknowledgments

Highly acknowledge is Austrian FFG and Polish NCBR for funding the "jawImplant" project in the frame of the European H2020 "M.ERA-NET" initiative.

MODIFICATION OF TITANIUM IMPLANT SURFACE FOR ANIMALS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 16]

Introduction

Animals are very specific and special patients. Time after material implantation to animal bone is very important. It is difficult to treat animals infection. On the other hand, for some owners it is difficult to appropriate dose an antibiotic for their animals. Modification of the titanium implants surface may enhance a osseointegration process and reduce time to return an animal to their daily activities. One of the technique for the surface treatment is plasma electrolytic oxidation process. The result of titanium implant treatment is formation a porous oxide layer on a metal surface [1-3]. The oxide layer is enriched in bioactive calcium and phosphorous compounds. However, the oxide layer does not protect against bacteria. To add antibacterial properties, on the oxide layer a thin polymer coating is formed using dip-coating technique. A polymer layer, such as poly(adipic sebacine) is deposited on the implant surface with amoxicillin. The aim of this work is formation the oxide-polymer layer on the titanium bone wedge implants. The layer should be cytocompatible and protect the surface against bacteria and formation bacteria biofilm.

Materials and Methods

Titanium implant (bone wedge, IWET, Poland) was anodized in solution composed of 0.1 M $Ca(H_2PO_2)_2$) at 300 V. Applied current density was 100 mA/cm^2 and time of the process was 5 min. Then, the anodized implant was immersed in 0.5% poly(sebacic anhydrite) solution with 5% (w/w) amoxicillin in trichloromethane with controlled immersion and withdraw speed: 100 mm/min. The surface of the implant was analysed using scanning electron microscope (Phenom ProX), non-contact optical profilometer (Wyko N9300, Veeco). Surface wettability was analysed using a goniometer (DataPhysics OCA 15EC). Drug release and drug loaded into the coating was analysed using high-performance liquid chromatography (Shimadzu, LC2030C Plus Prominence-i). Cytocompatibility of the polymer and antibiotic was evaluated using a mouse fibroblast L929 cells.

Results and Discussion

The porous oxide layer was formed on whole titanium implant surface. The layer was formed also on the corner of the implant. Polymer layer was formed on the previously anodized implant surface using a dip coating technique. Contact angle for only anodized implant surface was 31.1° ± 2.8, for implant with oxide-polymer layer was lower: 19.2° ± 2.7. Average surface roughness (Ra) for the only anodized implant (FIG. 1A) was 2.20 µm ± 0.15, whereas for the implant with oxide-polymer layer the Ra was 1.13 µm ± 0.12 (FIG. 2A).

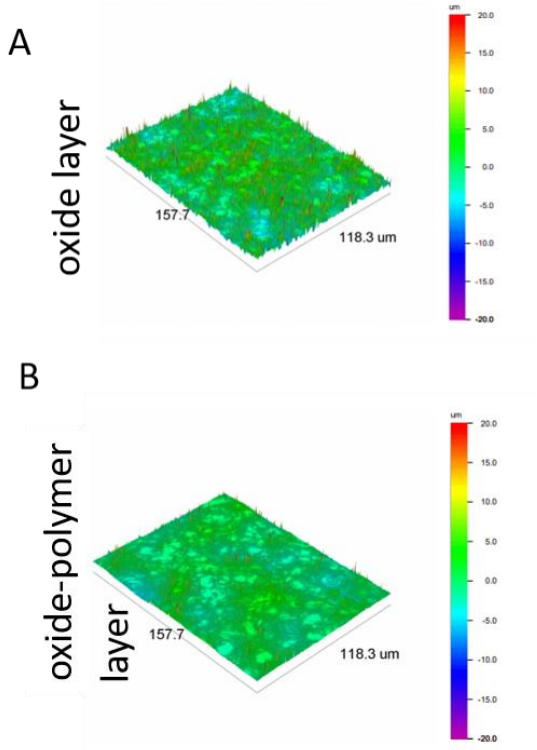

FIG. 1. Images of surface roughness of the samples form a selected area of a) oxide layer, b) oxide-polymer layer formed on a bone wedge.

HPLC analysis was applied to determined total loaded amoxicillin in polymer coating, and it was 79.68 µg/cm^2 of the bone wedge. Drug release from modified implant surface was analysed in Ringer solution, and after 30 min of the implant immersion concentration of amoxicillin was 191.69 µg/mL After 8h of implant immersion concentration of amoxicillin in Ringer solution increased to 203.00 µg/mL It was a 16.44% of the total loaded amoxicillin in polymer deposited on previously anodized bone wedge. Cytocompatibility test showed that concentration of amoxicillin up to 150 µg/mL does not significant decrease cell viability. Extract of the polymer used in this experiment is not toxic for the cells when the concentration does not exceed 1.5 wt.%.

Conclusions

Titanium bone wedge was anodized in solution with bioactive compounds. The polymer layer deposited on anodized Ti implant was loaded with amoxicillin with concentration up to 80 µg/cm^2. The concentration of loaded drug is not cytotoxic for the L929 cells. The drug is release from the coating in relatively short time, and it is concentration could be favourable to prevent bacteria adhesion and formation biofilm on the implant surface.

Acknowledgments

This work was supported by the National Centre for Research and Development, Poland, according to the LIDER XI programme. Project "Technology of hybrid coatings formation on titanium implants for animals" no. LIDER/12/0048/L-11/19/NCBR/2020.

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EFFECT OF SODIUM ALGINATE/POLY(VINYL ALCOHOL) RATIO AND *ALOE VERA* EXTRACT AMOUNT ON SELECTED CHARACTERISTICS OF HYDROGEL MATRIX INTENDED FOR BIOMEDICINE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 17]

Introduction

The wound healing mainly depends on the use of proper dressing techniques and adequate material. As maintaining an optimal moisture level and proper gas circulation as well as the ability to absorb excess exudate by the dressing, hydrogels are currently the most widely studied group of chemical compounds used in a new generation of wound dressing systems [1,2]. Alginate hydrogels constitute a group of materials which are applied to the skin in case of wounds with difficulty healing, as gelation of alginates ensures less noticeable pain while the dressing changes and the moist environment leads to rapid wound healing. In turn, poly(vinyl alcohol) possesses desirable properties such as nontoxicity, biocompatibility, chemical and mechanical resistance, and it exhibits high hydrophilicity with a high degree of hydrolysis. Currently, there is also noticed a trend to modify the hydrogel matrix with substances of natural origin, mainly extracted from plants. *Aloe vera* is one of the plants widely used in medicine, due to a broad health-promoting properties [1-4].
The presented paper focuses on the analysis of the effect of sodium alginate (SA)/poly(vinyl alcohol) (PVA) ratio and *Aloe vera* (AV) extract amount on selected physicochemical and structural properties of hydrogel matrices as a base for further modifications which will eventually lead to design the modern dressing materials.

Materials and Methods

In order to obtain SA/PVA/AV hydrogels through chemical crosslinking method, 2% SA, 5% PVA, 2% *Aloe vera* extract and 1% persuphate ammonium solutions were prepared. The appropriate amounts of such-prepared solutions, as well as constant amount of poly(ethylene glycol) diacrylate (PEGDA, Mn=700 g/mol) and glycerine (G) were utilized. The series of hydrogels with varied SA/PVA ratios (1:1; 2:1; 3:2, v/v) and different *Aloe vera* contents (1, 5, and 10%, v/v) were prepared. The analyses of the resultant hydrogels included determination of the swelling behaviour, degradation tests in distilled water and PBS solution at 37°C, temperature close to human body temperature. Moreover, the matrices were subjected to spectroscopic (FTIR) and microscopic (SEM) analysis.

Results and Discussion

A significant effect of the volume ratio of the main components of the tested hydrogel matrices on the behaviour in simulated body fluids was observed. The study showed that an increase in the proportion of alginates in the matrix leads to an increased and abrupt absorption of the fluid, resulting from the unique absorption properties of this type of materials. On the other hand, decreasing the SA content in the matrix provides a smooth and gradual swelling process, which suggests that any introduced active substances will be absorbed and released from the matrix in a controlled manner. It appears that the SA/PVA ratio, 1:1 appears to be the best choice for the characteristics studied. An example of the swelling ratio is presented in FIG. 1. The matrices with high plant extract content behave similarly - 10% content causes sudden and dynamic changes in the degree of swelling, pH and conductivity of the materials. It can negatively influence the prediction of therapeutic substances released from such matrices. Lower contents of *Aloe vera* extract cause more static change of tested parameters.
The FT-IR analysis confirmed the presence of absorption bands characteristic for all used components and microscopic images indicate the successful crosslinking of the materials.

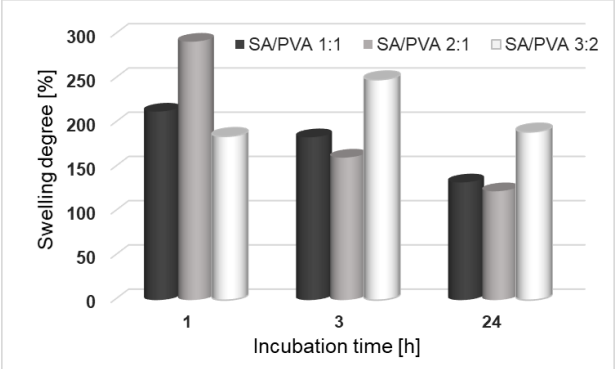

FIG. 1. The swelling degree in PBS solution of the SA/PVA/AV hydrogels with 5% of aloe amount and varied SA/PVA ratio.

Conclusions

The results obtained allow to draw the following conclusions:

1. Obtained hydrogel materials are characterized by a comparable level of swelling degree, however, the higher SA content and applying the 10% of aloe extract caused we observed that the structure is softer and characterized by low gel strength and therefore, the modified matrix can absorb a higher amount of fluid.
2. The degradation in water and PBS solution investigations allow concluding that the obtained SA/PVA/AV hydrogels are, in general, stable in time and pH value mainly depends on aloe extract content.
3. On the basis of FT-IR spectra of samples modified with different amounts of the extract, we noticed that their chemical structure is not influenced directly, even with 10% of extract content.
4. In accordance with presented preliminary analysis of physicochemical properties, it can be statement that the obtained materials can be used for further studies on a new approach to dermal wound healing.

Acknowledgments

The research was financed by The National Centre for Research and Development in the frame of the 41/0146/L-9/17/NCBR/2018 project.

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SYNTHESIS OF BACTERIOSTATIC POLYLACTIDE BY USING ZIRCONIUM (IV) AND ZINC (II) CHELATE COMPLEXES WITH AMINO-ACIDS BASED LIGANDS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 18]

Introduction

One of the big risks that may be overlooked in the current viral pandemic is the increase in disease caused by drug-resistant bacteria. Metal complexes (metallodrugs) look like an excellent alternative to classical antibiotics for treating diseases caused by bacteria [1]. Such a compound should demonstrate a strong antibacterial and antifungal activity on a large spectrum of microorganisms, and at the same time, low toxicity.
Compounds of this type are zinc and zirconium complexes containing ligands based on Schiff's bases. By appropriate selection of such a compound, it is possible to obtain metal complexes with bactericidal properties and being a good initiator of ROP. By using this kind of complex can allow to obtain biologically active biodegradable polymers with uniform morphology and containing an antibacterial complex in the ideal dispersed molecular form, which is practically impossible to obtain by blending bioactive substance in a high-molecular polymer.

Materials and Methods

Zinc (II) acetylacetonate monohydrate (Alfa Aesar, USA), Zirconium acetylacetonate L-tryptophan, L-phenylalanine, 4-pyridinecarboxaldehyde, methanol anhydrous 99.8%, benzene anhydrous 99.8%, chloroform anhydrous 99%, tetrahydrofuran anhydrous 99.9% was purchased from Sigma - Aldrich, Poland, and potassium hydroxide reagent grade was received from Merck. All these chemicals were used as received.
L-lactide (Forusorb, medical-grade) was received from Foryou Medical Device Co., Ltd. China and before use was purified by recrystallization from dry ethyl acetate.
Schiff base ligands HPhe and HTrp were synthesized using a previously reported method [2]. Zinc complexes; $Zn[(acac)(LPhe)H_2O]$, $Zn[(acac)(LTrp)H_2O]$ and Zircon complexes $Zr[(acac)_3(LPhe)]$, $Zr[(acac)_3(LPhe)]$ obtained by the method, was a modification of the previously published [3]. The lactide polymerization process was investigated under bulk conditions at 120°C with different contents of the zinc and zirconium initiators (M/I molar ratio as; 150:1, 400:1, and 600:1).
Estimation of the antibacterial and antifungal activities of the tested samples was done using a microtiter broth dilution method, as recommended by the Clinical and Laboratory Standards Institute [4]. In vitro cytocompatibility of polymeric materials and initiators was studied using the human normal CCD-11Lu fibroblast cell line (ATCC; CCL-202).
The conversion of the reaction and structure of obtained products was determined with NMR spectroscopy (Bruker Avance IITM 500 MHz at 25°C in DMSOd6).
The number-average and weight-average molar masses of the oligomers were determined by gel permeation chromatography with a Viscotek RImax chromatograph (Malvern Panalytical Ltd). FTIR spectra were recorded on JASCO FTIR-6700. The percentage of carbon, hydrogen, and nitrogen in the complex samples was determined by the VARIO EL III Element analyzer. All geometric structures of the zinc and zirconium complexes were fully optimized at the B3LYP/6-311G* density functional (DFT) level by using the Gaussian 03 Rev. E.01-SMP program [5].

Results and Discussion

Polylactide was obtained by ring-opening polymerization of lactide initiated with selected low-toxic zinc and zircon complexes $Zn[(acac)(L)H_2O]$ or $Zr[(acac)_3L]$ where L represents N-(pyridin-4-ylmethylene) tryptophan or N-(2-pyridin-4-ylethylidene) phenylalanine. These initiators were obtained by reaction of $Zn[(acac)_2H_2O]$ or $Zr(acac)_4$ with previously synthesized Schiff bases, the product of the condensation of amino acids and 4-pyridine carboxaldehyde. Both zinc complexes showed the geometry of a distorted trigonal bipyramid. Zirconium complexes presented a square antiprismatic form. Virtually all of these complexes as initiators of L-lactide polymerization showed high efficiency and made it possible to obtain high molecular weight polylactide. All these complexes were much more active in polymerization compared to the starting acetylacetonates. The synthesized high molecular polylactide (M/I molar ratio as 1:400) showed antibacterial properties, especially the product obtained by polymerization initiated by a zinc(II) complex with ligand-based on L-phenylalanine. The obtained polylactide quite unexpectedly showed a particularly strong antimicrobial effect against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Aspergillus brasiliensis*. At the same time, this polymer shows biocompatibility, does not exhibit fibroblast cytotoxicity.

Conclusions

By using selected non-toxic zinc or zirconium complexes showing a strong antibacterial effect and being effective ROP initiators, it is possible to obtain bioresorbable polymers showing significant antibacterial and antifungal activity.

Acknowledgments

This research was funded by NATIONAL SCIENCE CENTER POLAND, grant number UMO-2019/33/B/ST5/00743 “Bioresorbable polymers and polymer blends with bactericidal properties for use in cosmetics and dermatology”.

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ADVANCED BIO-HYBRID MATERIALS INCORPORATED WITH THE NANOCARRIER-DRUG SYSTEM AS MULTI-COMPARTMENTAL DRESSINGS SUPPORTING THE TREATMENT OF *PSORIASIS*

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[ENGINEERING OF BIOMATERIALS 163 (2021) 19]

Introduction

The proposed research solution addresses issues related to *Psoriasis* treatment problems. So far, no effective way to treat this disease has been found. It is estimated that it covers 2-5% of the world's population, representing approximately 140-210 million patients. Various types of ointments are most commonly used in the topical treatment of psoriasis. They include corticosteroids (predominantly betamethasone dipropionate), analogs of vitamin D3 (calcipotriol, calcitriol or takalcytol), tar, retinoids (e.g. tazarotene) or calcineurin inhibitors (tacrolimus, pimecrolimus). A significant drawback of this type of skin care is the necessity of frequent application of the drug, low effectiveness and often short-lived effect [1-4]. Last time the tendency to design and obtain new modified hydrogels containing the combination of the natural and synthetic active substance for medical applications, was observed [5-7].

Materials and Methods

Here, studies were focused on the design and development of bio-hybrid hydrogels based on sodium alginate (SA), poly(vinyl alcohol) (PVA), glycerine and *Aloe vera* solution (AV). Additionally, salicylic acid and fluocinolone acetonide-thermosensitive nanocarrier were incorporated into SA/PVA/AV hydrogel matrix. The bio-hybrid hydrogels were obtained through the chemical crosslinking method using poly(ethylene glycol) diacrylate (PEGDA, M_n = 700 g/mol) as a crosslinking agent [8].
After that, the chemical structure of the obtained bio-hybrid hydrogels was confirmed using FT-IR spectroscopy. The morphology was analyzed based on SEM microphotographs. Additionally, the physicochemical properties, such as gel fraction, swelling behaviour and degradation in distilled water and simulated body fluids, were carried out. In the next step, the release profiles of selected drugs, were determined. Finally, the cytotoxicity tests using *in vitro* method were conducted.

Results and Discussion

On the basis of the results, we can conclude that the presence of the nanocarrier-drug system does not influence significantly on the physicochemical and structural properties. Generally, the bio-hybrid matrix is characterized by very similar parameters in comparison to the basic matrix without drugs, which is a positive aspect. Moreover, SEM images exhibit the presence of the nanocarrier-drug system in the inside of the bio-hybrid hydrogel structure (FIG. 1).

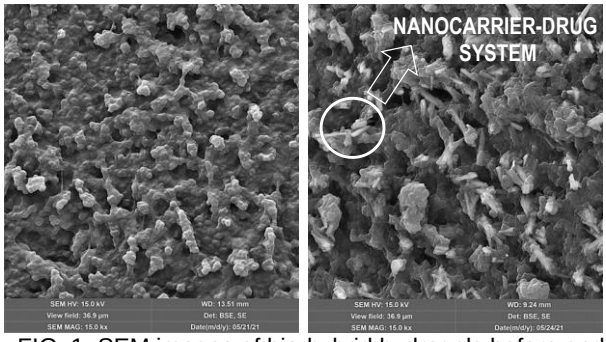

FIG. 1. SEM images of bio-hybrid hydrogels before and after modification.

Conclusions

The main goal of the research was the development of an effective method of the preparation of transparent bio-hybrid hydrogels containing salicylic acid and fluocinolone acetonide - thermosensitive nanocarrier system, which was achieved successfully. Moreover, this solution allows obtaining a double system, in which the release time of drugs is prolonged significantly, even up to 7 days.

Acknowledgments

This research was financial supported by The National Centre for Research and Development - project LIDER/41/0146/L-9/17/NCBR/2018.

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CURCUMIN LOADED P3HB-MCNTS ELECTROSPUN SCAFFOLDS – IN VITRO AND IN VIVO STUDY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 20]

Introduction

Electrospinning has recently attracted attention to create different nanofibrous structures emulating morphology of the extracellular matrix (ECM)[1]. Bacteria-synthesized biopolymers, such as poly(3-hydroxy butyrate) (P3HB), have triggered special attention in the development of various medical devices, soft and hard tissue engineering scaffolds and drug delivery systems[2]. However, P3HB electrospun scaffolds are not free from problems, as their lack of mechanical strength is a significant limitation for hard tissue engineering applications [1]. Therefore, different additives, such as functionalized multiwalled carbon nanotubes (MCNTs), can be introduced to improve not only mechanical properties, but also the biological response [1,3]. Another candidate with good anti-inflammatory and anti-oxidant properties is curcumin (CUR), which has been selected for reducing inflammation in different studies [4]. CUR has also been studied in combination with different polymers to produce electrospun scaffolds[5]. With this in mind, we combined P3HB-MCNTs for the first time with CUR to reduce the possibility of inflammation of electrospun scaffolds in their use as potential structures for bone tissue engineering applications.

Materials and Methods

P3HB powder was dissolved in chloroform/ dimethylformamide (7/3 v/v), MCNTs (0.5%wt) and CUR (10 and 20%wt) were added to the solution and loaded into the syringe for electrospinning device. The scaffolds morphology was evaluated using scanning electron microscopy (SEM) after 4 weeks immersing into the SBF (bioactivity) and PBS solution (biodegradability). Mesenchymal stem cells (MSCs) were cultured for 10 days (*in vitro* biocompatibility) and subcutaneous implantation of the scaffolds (*in vivo* biocompatibility) has been performed for 8 weeks in rat animal model.

Results and Discussion

The appearance of hydroxyapatite (HA) crystals on the surface of the fibers indicated the bioactivity of the scaffolds[6] (FIG. 1). The amount of sedimentary HA on the scaffolds with 20% of CUR was the highest indicating high bioactivity.

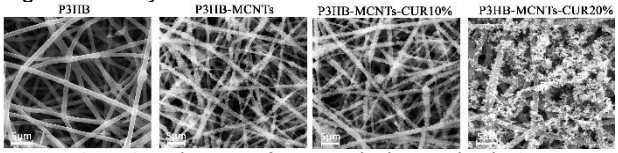

FIG. 1. SEM images after 4 weeks immersing in SBF.

FIG. 2 shows SEM pictures of the cultured cells attached onto the scaffolds after ten days (FIG. 2A) and the results from MTT assay at the first, fifth, and tenth day of seeding the MSCs cells onto the scaffolds (FIG. 2B). An increase in cell viability has been noticed for scaffolds containing 10wt% CUR, and significantly higher cell viability was observed for scaffolds loaded with 20% CUR.

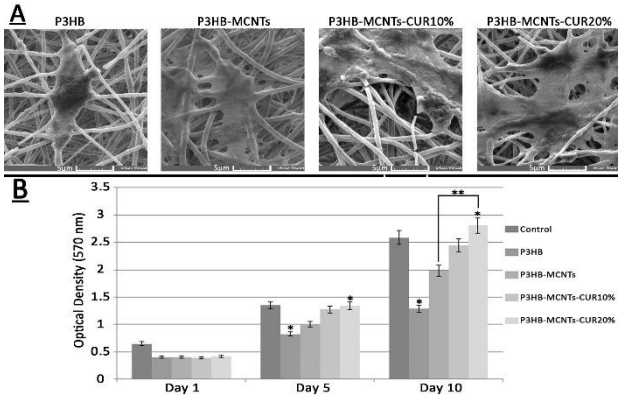

FIG. 2. SEM images of cell attachment onto the surface of the fibers (A) and MTT results (B)

The addition of 20% CUR increased the biodegradation rate to about 35% of mass loss after 4 weeks (FIG. 3). SEM micrographs of the scaffolds structure clearly indicate changes on the surface of the fibers.

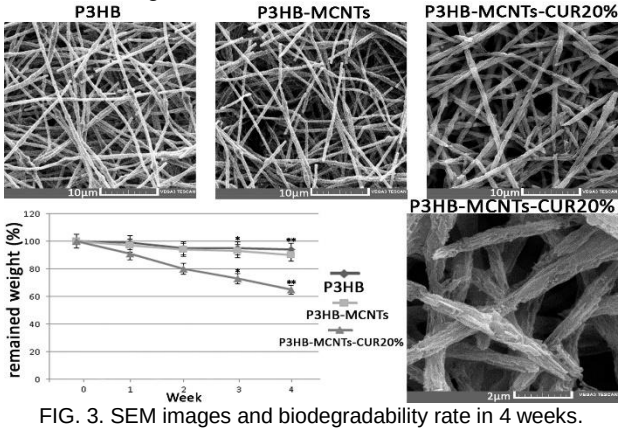

FIG. 3. SEM images and biodegradability rate in 4 weeks.

Scaffolds loaded with 20% of CUR were still present after 8 weeks of implantation (FIG. 4). Notably, there was less acute inflammation due to the presence of CUR as well as higher resorption of scaffolds. Some vessel formation around the scaffolds can also be seen.

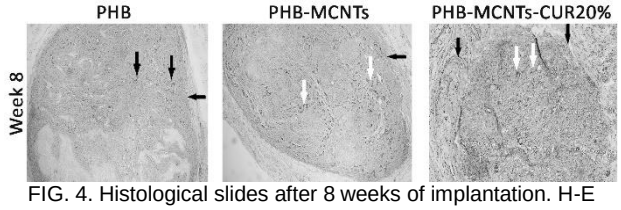

FIG. 4. Histological slides after 8 weeks of implantation. H-E staining (white arrows: foreign body type giant cell reactions and blue arrows: vessel formation around the scaffolds).

Conclusions

Composite scaffolds containing MCNTs and CUR showed accelerated hydrolytic degradation in PBS and enhanced hydroxyapatite precipitation from SBF as compared to neat P3HB. Moreover, CUR strongly reduced inflammatory reaction after 8 weeks of *in vivo* implantation of the scaffolds. Overall, our findings clearly indicate that electrospun scaffolds made of P3HB-MCNTs-CUR20% can be promising structures for tissue engineering applications.

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DESIGN OF INITIAL FIXATION IN BONE OF CUSTOMIZED INNOVATIVE HIP RESURFACING ENDOPROSTHESIS BASED ON VALIDATED NUMERICAL MODEL OF THE BIOMIMETIC MULTI-SPIKED CONNECTING SCAFFOLD EMBEDDING IN BONE AND DATA FROM THE HUMAN OSTEOARTHRITIC FEMORAL HEADS μCT ASSESSMENT

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[ENGINEERING OF BIOMATERIALS 163 (2021) 21]

Introduction

The prototype multi-spiked connecting scaffold (MSC-Scaffold) is a new kind of biomimetic fixation for resurfacing endoprostheses [1-3]. This scaffold mimics the natural interface between articular cartilage and subchondral trabecular bone in human joints. The numerical model of the MSC-Scaffold embedding in bone developed in previous studies [4,5] enables simulation of load transfer from the MSC-Scaffold to periarticular bone. The assisted by computed microtomography (μCT) examination of mechanical embedding of the MSC-Scaffold prototypes in periarticular bone has provided insight into the mechanical behaviour of considered system, and allowed for validation of this model [5,6]. Further μCT assessment of subchondral trabecular bone before and after the MSC-Scaffold embedding in human osteoarthritic femoral heads has revealed densification of bone microarchitecture caused by the MSC-Scaffold embedding [7]. Based on the above findings it was attempted to design of initial fixation in bone of customized innovative hip resurfacing endoprosthesis applying the validated numerical model and data from the human osteoarthritic femoral heads μCT assessment.

Materials and Methods

For the design of initial fixation in bone of customized innovative hip resurfacing endoprosthesis there was applied the numerical model validated in μCT-assisted mechanical tests performed on fresh swine femoral heads [5,6]. The subchondral trabecular bone relative area (BA/TA) was measured on binarized images obtained during the uCT assessment (GE phoenix v|tome|x s240) of bone microarchitecture before and after the MSC-Scaffold embedding in human osteoarthritic femoral heads. Then, to determine the mechanical properties of this bone, Young's modulus of bone in the MSC-Scaffold embedding direction E_2 was calculated based on the known from BA/TA measurements apparent bone density ρ, according to the empirically established formula: $E = 1.310\rho^{1.40}$ [8].

Results and Discussion

In TABLE 1 there are presented the calculated values of Young modulus in embedding direction E_2 of subchondral trabecular bone at different levels of the MSC-Scaffold embedding.

TABLE 1. Young modulus in embedding direction E_2 of bone at different levels of MSC-Scaffold embedding.

Level of MSC-Scaffold embedding [mm]	1.0	2.0	3.0	4.0
Young modulus in embedding direction E_2 [MPa]	842	964	1096	1172

FIG. 1 shows the force-embedding level relationship from simulations applying the validated numerical model of the MSC-Scaffold embedding in bone and values of Young modulus in embedding direction E_2 of subchondral trabecular bone, calculated based on μCT assessment of human osteoarthritic femoral heads given in TABLE 1.

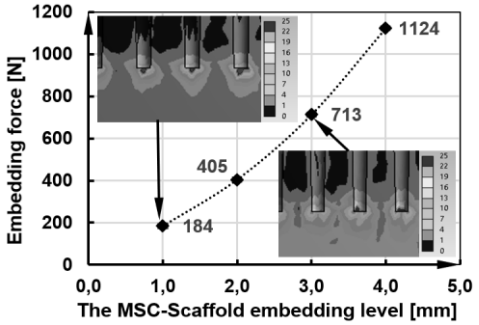

FIG. 1. The force-embedding level relationship from the simulation study of the MSC-Scaffold embedding in bone. Insets show maps of the Huber–von Mises–Hencky stress distribution in peri-impant bone for the considered MSC-Scaffold embedding level.

As seen in FIG. 1, the embedding force increases significantly with the MSC-Scaffold embedding level. This is due to the following two factors: the increase in the contact area between spikes of the MSC-Scaffold and the peri-implant bone, and the peri-implant bone material densification. The obtained results allow to assess efficient embedding level for the MSC-Scaffold initial fixation in human bone of patient with OA, allowing safe postoperative limb loading. Furthermore, based on pre-operative data from QCT assessment of bone of particular patient the geometric features of the MSC-Scaffold can be designed as customized for this patient.

Conclusions

The validated numerical model of the MSC-Scaffold embedding in bone is useful in the bioengineering design of the initial fixation of the customized innovative resurfacing endoprostheses with the biomimetic MSC-Scaffold.

Acknowledgments

Authors are grateful to M. Dąbrowski PhD MD and the Poznan University of Medical Sciences for granting the research project, with the approval of the Bioethics Committee (No. 146/2018).

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MAGNETIC NANOPARTICLES FOR CANCER CELLS CAPTURE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 22]

Introduction

Metastasis is the leading cause of cancer-related deaths; thus, its early detection is crucial for patient's prognosis and optimal treatment strategy. The detachment and migration of the cells from the primary tumor is enabled by the change in their phenotype, known as the epithelial-mesenchymal transition (EMT). EMT permits the epithelial cells to go through a series of biochemical and morphological processes, shifting them to the more invasive, mesenchymal phenotype with enhanced migratory properties [1]. Such cells, known as circulating tumor cells (CTC), can spread cancer to distant locations. Capturing, and analysing the captured CTC allows for a better estimation of patient's prognosis, and may allow to prevent, limit, or at least slow down metastasis. Among various approaches to this problem the use of inorganic or hybrid magnetic nanoparticles seems quite promising. Here we report on two different systems: targeted superparamagnetic iron oxide nanoparticles (SPION) stabilized with chitosan derivative and decorated with anti-N-cadherin antibodies and halloysite nanotubes modified with SPION. Both systems were designed to bind CTC, allowing for their magnetic capture, removal from the bloodstream and subsequent analysis.

Materials and Methods

Chitosan was modified using glycidyltrimethylammonium chloride (GTMAC, ≥ 90%, Sigma-Aldrich) according to the procedure developed earlier [2]. SPION were obtained by co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of cationic derivative of chitosan (CCh). For the flow cytometry studies nanoparticles were fluorescently labelled with fluorescein isothiocyanate (BioReagent, Sigma-Aldrich). Halloysite nanotubes (nanopowder; 30-70 nm dia × 1-3 μm length) were modified with SPION also using the co-precipitation method, however, the OH^- ions were produced *in situ* by urease (from *Canavalia ensiformis*, type C-3, powder, ≥600,000 units/g solid) in the presence of urea. The enzyme was entrapped inside the nanotubes' lumen before the modification step.

The systems obtained were characterized using DLS, ATR-FTIR, and SEM/STEM. The magnetic properties of the nanoparticles were measured using magnetometry and Mössbauer spectroscopy. The targeted SPION were also studied using confocal microscopy, AFM and flow cytometry. Human prostate cell lines (American Type Culture Collection—ATCC, Manassas, Virginia, USA) - LNCaP PC-3 and DU 145 were cultured in supplemented RPMI 1640 medium with 10% (v/v) of FBS in the incubator (37ºC, 90% humidity with 5% CO_2) according to previously described procedure [3]. Cytotoxicity measurements were performed using MTT and Alamar Blue assays.

Results and Discussion

SPION/CCh nanoparticles were decorated with anti-N-cadherin antibodies, which were specific to N-cadherin present on the surface of cancer cells which underwent EMT. The nanoparticles obtained had an average size of ca. 30 nm, as measured by AFM. They also had a moderate tendency to form aggregates, which had an average hydrodynamic diameter of 223 nm and were colloidally stable (zeta potential of – 45 mV). The magnetic properties of the nanoparticles were excellent, providing the superparamagnetic character of the system. Flow cytometry experiments allowed us to find out if SPION/CCh-N-cad bind to cancer cells expressing N-cadherin and how fast the binding process is. Confocal microscopy allowed us also to see the distribution of the nanoparticles within the PC-3 monoculture and the co-culture with LNCaP cells (before EMT, low N-cadherin expression). Magnetic capture experiments in quasi-static system (specially designed and 3D-printed cuvette) were performed. To enhance the magnetic effect, we have also obtained halloysite nanotubes loaded with SPION. The synthesis was based on the procedure proposed by Zheng et al. [4]. Urease/urea system was used as a source of hydroxyl groups, allowing to form SPION in situ. near the inner surface of the nanotubes. To obtain smaller particles with more uniform size the halloysite was pre-treated using high-power sonication in the presence of a surfactant (hexadecyltrimethylammonium bromide, CTAB, ≥99.0%, Sigma-Aldrich). The pre-treated halloysite had an average hydrodynamic diameter of 315 nm and relatively uniform size (PDI = 0.183). Magnetic halloysite was easily separated from the suspension with a magnet. The preliminary microscopic studies (SEM/EDX) confirmed the formation of the nanoparticles of iron oxide.

Conclusions

SPION/CCh decorated with anti N-cadherin antibody were found to be a colloidally stable system. The magnetic properties of the SPION were not affected by the coating and modification with antibodies. Flow cytometry confirmed the effective magnetic capture of PC-3 cells with bound SPION/CCh/N-cad particles. The incubation time as short as 1 minute was sufficient for SPION/CCh/N-cad to effectively bind to cancer cells. Confocal microscopy images of co-cultures confirmed the specificity of interactions of SPION/CCh/N-cad system with N-cadherin expressing PC-3 cells. SPION were also successfully deposited on the surfaces of halloysite nanotubes, rendering them magnetic and prospecting for CTC capture.

Acknowledgments

Karolina Karnas acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16. Joanna Dulińska-Litewka, Anna Karewicz, and Czesław Kapusta acknowledge the support of Narodowe Centrum Nauki, grant no. 2020/39/B/NZ5/03142.

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MULTINUCLEAR TITANIUM(IV)-OXO COMPLEXES AS THE NOVELTY ANTIMICROBIAL AGENTS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 23]

Introduction

Excessive and misuse of antibiotics and antimicrobials led to a spread of microorganisms resistant to most currently used agents. The resulting global threats became an impulse in searching for new materials with optimal antimicrobial activity and their application in various areas of our lives. Our research focused on the formation of composite materials produced by the dispersion of multinuclear titanium(IV)-oxo complexes (TOCs) in polymer matrices, which exhibit optimal antimicrobial activity.

Materials and Methods

The TOCs, of the general formula $[Ti_aO_b(OR)_c(O_2CR')_{4a-2b-c}]$ (R = ^{i}Pr, ^{i}Bu; R' = $PhNH_2$, PhOH, and $C_{13}H_9$) were isolated from the mother liquors consisted of $Ti(OR)_4$ and organic acids (HO_2CR') accordingly procedure earlier described [1-3]. Analysis of X-ray diffraction data and spectra registered using diffuse reflectance infrared Fourier transformation (DRIFT) and Raman spectroscopy allowed on their structure confirmation [1-5]. The isolated TOCs microcrystalline powders were dispersed in the polymer matrices (PMMA, PCL, and epoxide resins), thus forming composites (polymer + *n*TOCs), containing *n* = 2, 5, 10, and 20wt% of the oxo-complex. The presence of TOCs in (polymer + TOCs) composites and their structural stability during the fabrication of composite samples has been confirmed by the registration of Raman microscope maps and Energy dispersive X-ray spectroscopy (SEM EDX). Moreover, the possible changes in thermal properties of studied composites, caused by the addition of TOCs, were estimated using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The photocatalytic activity was estimated on the basis of methylene blue solution (MB) decolorization, during the irradiation with visible light by 30 h. EPR spectroscopy was used to detect paramagnetic species on the surface of the synthesized materials. Antibacterial and antifungal activities of composite samples was studied against Gram-positive (*Staphylococcus aureus* ATCC 6538 and *S. aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 8739 and *E. coli* ATCC 25922) bacteria and yeasts of *Candida albicans* ATCC 10231.

Results and Discussion

he results of our investigations allowed us to develop methods for the synthesis of multinuclear Ti(IV)-oxo complexes (TOCs) with different $\{Ti_aO_b\}$ core architecture, as well as their structural characteristic [1-5]. Obtained results proved the hydrophobic nature of TOCs crystals and powders; additionally, they revealed a low sensitivity to hydrolysis processes. It caused that in all our photocatalytic and biological experiments, the (polymer + TOCs) composites produced by the TOCs dispersion in the polymer matrix, were used. The preliminary spectral (IR, Raman) investigations confirmed the structural stability of TOCs during the composite sample production and the photocatalytic experiments. Moreover, we have noticed a clear relationship between the $-O_2CR'$ group type, stabilizing the oxo-core, and TOCs photocatalytic activity. It should be noted that in all our experiments, the samples were illuminated with UV irradiation (UVB-UVA range). However, in some cases, e.g. TOCs stabilized by the 9-fluorenecarboxylic or benzoic carboxylic species ligands, the broad absorption band also covers the visible range. In this case, it was possible to use visible light as a factor inducing photocatalytic processes. It suggests that the TOCs micro-grain samples excited by the visible light can generate the reactive oxygen species (ROS) as the potential microbicidal factors. The results of our preliminary studies on the antimicrobial activity of the (polymer + TOCs) composites (polymer = PMMA, TOCs = complexes containing $\{Ti_3O\}$ and $\{Ti_4O_2\}$) showed promising properties of this system as a microbiocidal agent [6,7]. Moreover, it was established that the antimicrobial activity of the (polymer + TOCs) composite was the result of the oxo-complexes introduction into the polymer matrix.

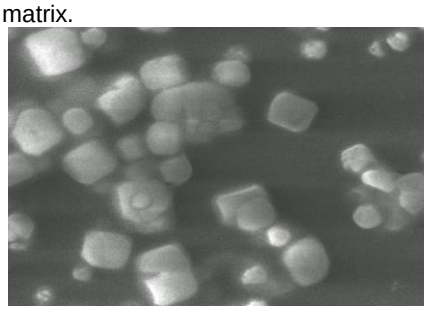

FIG. 1. The SEM image of the (PMMA + TOCs) composite sample.

Conclusions

The results of our investigations on TOCs synthesis, their physicochemical properties, and photocatalytical activity revealed that these compounds might be of vital importance for novel bioactive inorganic-organic composite materials formation. A significant was determining the dependency between the TOCs' structure, their photocatalytic activity in the visible light range, manifested by the generation of identified ROS, and their antimicrobial properties. Successful results of the future detailed microbiological tests and excluding cytotoxic properties of composites produced by dispersion of selected TOCs in polymer matrices will allow for designing new devices/coatings with the visible light-induced to solve differentiated problems related to the control of microbial pathogens.

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DESIGN AND MANUFACTURE OF CUSTOMIZED MEDICAL IMPLANTS - FINAL REPORT

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[ENGINEERING OF BIOMATERIALS 163 (2021) 24]

Introduction

In recent years, we reported herein about the progress of our project Program POIR 1/4.1.4/2017 financed by the National Center for Research and Development in Poland (NCBiR). The project concerned the design and manufacture of patient specific osseointegrated transcutaneous orthopaedic implants, intended for patients who have undergone an above the knee amputation. Such implants are implanted into the medullary cavity of a long bone and connected directly to an external prosthesis. As a result, loads can be transferred directly from the femoral stump to knee prosthesis and not through the soft tissues of the amputated limb, which is the case when using a socket-suspension type prosthesis system. A significant part of the planned research was carried out in line with the project schedule. However due to various reasons, at the end of 2020 the consortium was forced to make the decision to prematurely terminate the project. As a result of this the project did not enter the clinical stage. It is currently being evaluated by the NCBiR.

Materials and Methods

Implants were designed using reverse engineering, biomodelling and CAD software described in previous reports. Finite element analyses were performed for all of the implant prototypes and its connector. Prototypes were then manufactured form titanium alloy using a hybrid CNC milling system (Laser 1300, C.B. Ferrari, Italy). Sterilized implant prototypes were subjected to strength and fatigue tests in order to assess their suitability for long-term use. The sterilization technique used was the hot dry air method. Implant sterility was evaluated using microbiological tests. Thrombocompatibility was assessed using scanning electron microscopy and flow cytometry tests. Cytotoxicity was assessed by XTT test and genotoxicity by micronucleus test. The carcinogenic potential of the materials intended for the manufacture of the implants were also evaluated. For this purpose, qRT-PCR technology was used to analyse the expression of selected genes known to be involved in neoplastic processes. Additionally, cells were tested for proliferation potential, level of apoptosis and intensity of the DNA damage/repair process. The cells examined were primary and tumour osteoblast and chondrocyte lines.

Results and Discussion

Last year, we reported that the initial implant prototypes failed fatigue testing. The results of these tests were taken into account and several design changes to the implants were made. These new protypes were also manufactured from titanium alloy and consequently underwent fatigue testing. This time the protypes completed the entire fatigue testing procedure successfully.

The results of the work carried out, including sterilization validation, biological studies, implant design and in particular fatigue testing made it possible to initiate the final, clinical stage of the project. Although the clinical team prepared procedures for the surgical implantation process and had started recruiting patients to participate in the study, as mentioned above the project had to be terminated before any surgical procedures were performed.

Conclusions

A significant proportion of the project has been successfully completed with implant prototypes designed, tested and validated to the point where they could be applied clinically. Unfortunately, due to various factors including the ongoing COVID-19 pandemic and its significant impact on the health care system as well as the economy, the project had to be terminated before the clinical stage could be completed.

Acknowledgments

The project is financed by the National Center for Research and Development in accordance with the contract POIR.04.01.04-00-0058/17-00.

INFLUENCE OF CHITOSAN ORIGIN ON THE PROPERTIES OF ITS DERIVATIVES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 25]

Introduction

Chitosan is a natural polymer that can be obtained from crab, squid or shrimp shells, and we can also obtain it from mushrooms such as *Aspergillus niger*. Chitosan is insoluble in water, but it is biodegradable and non-toxic. One of the derivatives is carboxymethyl chitosan. Carboxymethyl chitosan (CMCS) is formed by the substitution of a carboxymethyl group for an amino group and/or a hydroxyl group. It has better physicochemical and biological properties than chitosan and is also water-soluble. [1-3] The purpose of this study was to investigate the influence of various chitosan origin on the properties of chitosan derivatives.

Materials and Methods

Chitosan from squid, chitosan from *Aspergillus niger* was purchased from POL_AURA. Sodium hydroxide, sodium chloride was received from POCH S.A. (Avantor, Poland). Chloroacetic acid Hydrochloric acid, Isopropyl alcohol was supplied Chempur (Poland). The method of synthesis was taken from the literature [1]

The obtained chitosan and chitosan derivatives were characterized using viscometric technique and infrared spectroscopy. The intrinsic viscosity of carboxymethyl chitosan in 0.1 mol/L NaCl aqueous solution at 30°C was carried out in an Ubbelohde capillary viscometer. For the chitosan samples the intrinsic viscosity was measured in 0.1 mol/L CH_3COOH/0.2 mol/L NaCl aqueous solution at 25°C. The viscosity average molecular weight was calculated according to the Mark–Houwink equation [4]. FTIR spectra of the used chitosan samples and chitosan derivatives were recorded on VERTEX 70v FT-IR Spectrometer (Brucker Optics Inc), in the wavelength range between 4000 – 400 cm^{-1}, resolution of 2 cm^{-1} and 60 – times scanning. The degree of substitution of each chitosan derivatives was obtained by potentiometric titration [5].

Results and Discussion

The synthesis products had a slightly yellow colour. Depending on the origin, the intensity of the colour varied. Obtaining N, O-carboxymethyl chitosan from both syntheses was confirmed based on spectroscopic analysis. This was confirmed by the appearance of characteristic peaks at wave numbers of 1590 cm^{-1}, 1410 cm^{-1}.1320 cm^{-1} (FIG. 1). The intrinsic viscosity of carboxymethyl chitosan solutions and the viscosity average molecular weight were determined based on viscometric measurements. It was also found that a large decrease in the molecular weight and GLL of catboxymethyl chitosans relative to chitosan (TABLE 1).

The resulting derivatives have varying degrees of substitution and average molecular weight.

Conclusions

The analysis show that the origin of chitosan influences the properties such as degree of substitution and viscosity average molecular weight of its N,O-carboxymethyl chitosans.

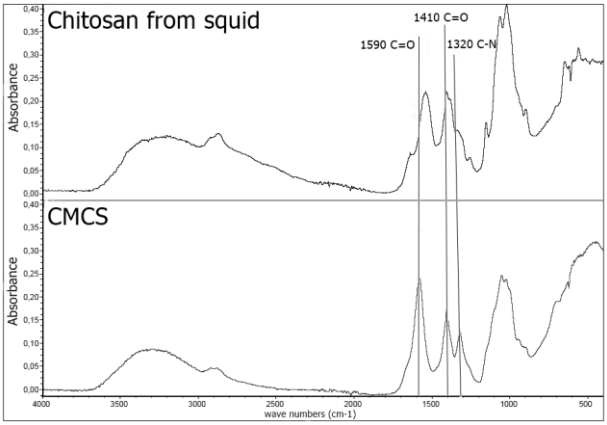

FIG. 1. FT-IR spectra of CMCS and chitosan from squid.

TABLE 1. Comparison of the viscosity average molecular weights and GLL of the CMCS and chitosan from squid.

Sample	GLL [cm^3/g]	Mv [g/mol]
Chitosan from squid	647	$9.36*10^5$
CMCS	237	$2.993*10^5$

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GOLD NANOPARTICLES AND SILICON AS EFFECTIVE MODIFIERS OF HYBRID-TYPE, CHEMICALLY BONDED BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 26]

Introduction

Calcium phosphate (CaP) bioceramics is widely used in the field of bone regeneration, mainly due to its excellent biocompatibility. Usually, CaPs-based biomaterials are produced in the sintered form as porous and dense blocks. The attractive alternative for pre-shaped bioceramics are chemically bonded materials in the form of mouldable pastes. Calcium phosphate-based bone cements (CPCs) are the most widely investigated group of these bone substitutes [1]. Recently CPCs enriched with granules and microspheres i.e., biomicroconcretes, have gained huge interest [2,3]. Granules when loaded with biologically active agents, can serve as delivery vehicles in targeted therapies. To obtain antimicrobial activity nanoparticles such as silver (AgNPs), copper (CuNPs), and gold (AuNPs) may be introduced into biomaterials [4]. Whereas, to improve biological performance, CaPs modified with elements such as silicon, magnesium or zinc can be developed [5]. A combination of these strategies can lead to production of biomaterials with some superior properties.
The aim of our study was to obtain and examine hybrid-type, chemically bonded biomaterials composed of surgically handy silicon-modified CPCs and hybrid AuNPs-loaded hydroxyapatite/chitosan granules.

Materials and Methods

The silicon-modified α-tricalcium phosphate (Si-αTCP) powder was used as a setting phase of chemically bonded biomaterials. Si-αTCP (0.3 wt.%Si) was synthesized by the wet chemical method. Hybrid hydroxyapatite/chitosan granules (HA/CTS) containing 17 wt.% chitosan (Sigma-Aldrich) modified with 0.1wt.% gold nanoparticles (US Research Nanomaterials), were synthesized via a wet chemical method. The solid phases of the biomicroconcretes were obtained by mixing the hybrid granules with α-tricalcium phosphate powder in a ratio of 2:3, respectively. The 0.75 wt.% methylcellulose solution in 2.0 wt.% Na_2HPO_4 was applied as the liquid phase of the biomicroconcretes.
The phase composition (XRD), microstructure (SEM, TEM), setting times (Gilmore needles), mechanical strength (Instron), and *in vitro* bioactive potential of the composites in simulated body fluid were examined. Furthermore, based on the AATCC 100, the antibacterial activity of the materials against *Staphylococcus epidermidis, Staphylococcus aureus* and *Escherichia coli* was evaluated.

Results and Discussion

The XRD analysis revealed that the biomicroconcretes composed of two major crystalline phases: α tricalcium phosphate and hydroxyapatite. The amorphous halo in XRD patterns refers to the presence of chitosan. SEM and TEM studies showed good adhesion at the granule/matrix interface (FIG. 1).

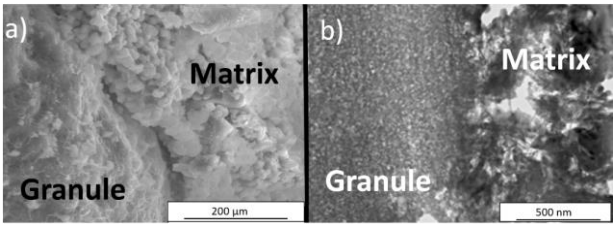

FIG. 1. SEM (a) and TEM (b) images of granule/matrix interface of AuNPs and silicon modified biomicroconcrete.

Furthermore, hybrid-type biomicroconcretes possessed acceptable setting times and mechanical properties appropriate for low-load bearing applications. The introduction of silicon and AuNPs led to a favourable shortening of the setting process (t_F = 10 min). Moreover, biomicroconcretes modified with gold nanoparticles and silicon possessed enhanced bioactivity, proven during *in vitro* studies in simulated body fluid. The *in vitro* tests revealed the antimicrobial activity of all developed biomicroconcretes against the tested bacterial strains i.e., *Staphylococcus epidermidis, Staphylococcus aureus* and *Escherichia coli*. This effect related to the presence of chitosan and gold nanoparticles.

Conclusions

The new hybrid-type, chemically bonded biomaterials based on hydroxyapatite and chitosan were successfully developed and investigated. Gold nanoparticles and silicon were proven to be effective modifiers of biomicroconcretes. Developed materials possessed physicochemical properties sufficient for non-load bearing applications. Furthermore, the bioactive potential as well as the antibacterial activity of all developed biomicroconcretes were confirmed in *in vitro* studies. The AuNPs and Si-modified composites were found to be promising candidates for further biological studies.

Acknowledgments

This research was funded by the National Science Centre, Poland (grant no. 2017/27/B/ST8/01173) and the Faculty of Materials Science and Ceramics AGH University of Science and Technology, Kraków, Poland (project no. 16.16.160.557).
Authors wish to thank Jerzy Morgiel from the Institute of Metallurgy and Materials Science, Polish Academy of Sciences, for conducting the TEM studies.

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FOAMED CALCIUM PHOSPHATE CEMENTS AND THEIR BEHAVIOUR DURING INCUBATION IN DISTILLED WATER

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[ENGINEERING OF BIOMATERIALS 163 (2021) 27]

Introduction

Bioactive, foamed calcium phosphate bone cements (fCPCs), obtained by surfactant-assisted foaming, are a relatively new group of bone substitutes[1]. Thanks to their porosity, these materials can act as scaffolds for bone tissue regeneration.

The selection of surfactants for the preparation of foamed bone cements is a very difficult issue. It is necessary to pay attention to many properties of the surfactant, such as its: chemical nature (ionic, non-ionic, etc.), hydrophilic-lipophilic balance value or solubility in water. The surfactant characteristics have a direct impact on the final materials properties, as can be seen from the previously described non-foamed cements [2].

In this study, the degradation behaviour of fCPCs was studied. The changes in water pH and ionic conductivity during materials incubation were investigated. The effect of 28-day immersion was evaluated by fCPCs microstructure observations using scanning electron microscopy (SEM). The release profile of the surfactants (Tween 20, Tween 80 and Tetronic 90R4) used for the cement foaming, was determined via high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS).

Materials and Methods

Alpha-tricalcium phosphate (α-TCP) synthesized by the wet chemical method was used as the solid phase of cements. As the liquid phase 2 wt.% Na_2HPO_4 aqueous solutions with selected surfactant (10 $g \cdot L^{-1}$) was used. The foaming agents - surfactants used in the study were: Tween 20, Tween 80 and Tetronic90R4 (Sigma Aldrich). The liquid to powder ratio (L/P) was equal 0.7 $g \cdot g^{-1}$. Cements fTW20, fTW80 and f90R4 were obtained by mixing the powder phase with a previously foamed surfactant solution (liquid phase). The control samples did not contain any surfactant (fCTRL). The cement samples were incubated in distilled water (1g of sample for 100 mL of water) at 37°C for 28 days. The measurements of ionic conductivity and pH were performed on days 0, 1, 3, 7, 14, 21, and 28 by pH/conducto-meter (Hanna H198129 Combo). SEM observations (NovaNano SEM) of materials were performed before and after 28-day incubation. In order to study the surfactants release from cement samples, after 3, 7, 14, 21 and 28 days, 300 µl of water was collected and stored at -20°C for subsequent analysis by HPLC-MS (Agilent 1290 Infinity, MS Agilent 6460 Triple Quad Detector).

Results and Discussion

Samples immersion led to increase of water ionic conductivity. Cements with polysorbates, i.e. Tween 20 and Tween 80 increased ionic conductivity more than cements without surfactant (fCTRL) or with Tetronic 90R4. The pH of water decreased along with the materials' soaking time. After 28-day incubation, on the cement surfaces, the presence of numerous apatite forms was observed (FIG. 1). The differences between the control sample (fCTRL) and those with surfactants was in the shape of apatite crystals, which was the needle-like and oval-like, respectively. In surfactant release study, for polysorbates (Tween 20, Tween 80) no significant changes in surfactant concentration after the third day of incubation was observed. The concentration of Tetronic 90R4 decreased up to day 7 and then reached a plateau.

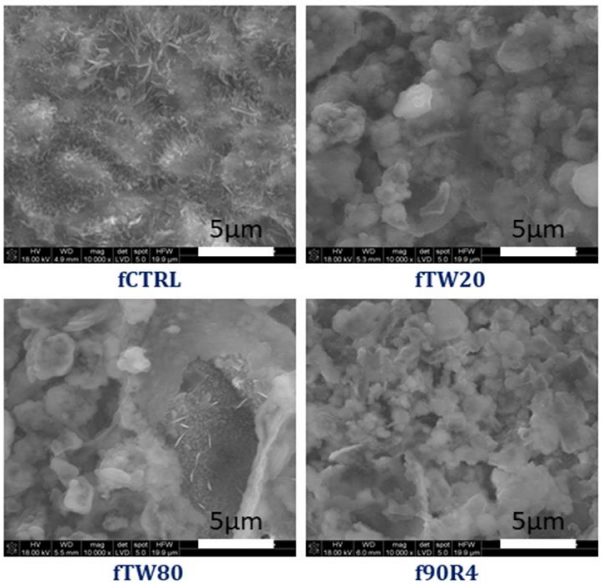

FIG. 1. Microstructure of cement surfaces after 28 days of incubation in distilled water.

Conclusions

Ionic conductivity can be related to the degree of cement foaming, which was much higher in the case of cements with Tween 20 or Tween 80. In addition, these surfactants degrading to their building fatty acids (lauric for Tween 20 and oleic for Tween 80) [3] contribute to the increase in ionic conductivity. On the surfaces of cements with surfactants, oval-shaped apatite crystals over plate- or needle-like morphologies were dominant, what stays in agreement with the results of our previous studies [4]. The release studies revealed that the surfactant concentration did not correspond to the theoretical value, i.e. the maximum amount that could be released from the material. Most likely, the released compounds had already degraded significantly before the third day of incubation. Obtained materials need further research.

Acknowledgements

This study was supported by the National Science Centre, Poland (Project No. 2017/27/N/ST8/00913). EC acknowledges financial support from the National Science Centre, Poland under Doctoral Scholarship No. 2019/32/T/ST5/00207. JP acknowledges the fellowship with the project POWR.03.02.00-00-I013/16.

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PHOTOSENSITIVE POLYMERIC MATERIALS DEDICATED TO LIGHTWEIGHT HEART PUMP ROTOR DESIGN

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[ENGINEERING OF BIOMATERIALS 163 (2021) 28]

Introduction

The innovative ReligaHeart ROT rotary implantable blood pump was developed. The chamber is in the preclinical research phase in patients with advanced myocardial dysfunction. It is a mechanical bearingless pump, equipped with a rotor suspended magnetically and hydraulically, which provides a flow of up to 10 l/min at 30-45% capacity. A fully magnetic rotor suspension system, without hydrodynamic bearings, is being developed to reduce shear stress on the blood and protect Von-Willebrand platelets and proteins from damage causing the risk of bleeding. ReligaHeart VASC, is currently under development and is designed for short-term cardiac support in cardiac shock. It has an implantable rotor system with a miniaturized motor and magnetic rotor suspension system. The objective of the task is to design a rotor taking into account the extended possibilities of creating structural properties of parts by SLA technology in comparison with conventional manufacturing, but also the limitations of SLA technology. The main reasons for the new 4Dblood ROT impeller design for the RH ROT pump resulted from the complex shape of the RH ROT pump impeller with hydrodynamic bearing blades. This design requires high precision impeller geometry for hydrodynamic bearing operation. In order to ensure the efficient operation of the hydrodynamic bearing in the pump design, it is necessary to maintain a distance of several hundredths of a millimetre between the impeller and the casing disc surface. The small distance between the rotor and the casing is a risk factor related to the danger of destruction of VonWilebrandt proteins by shear stress on the surface of the hydrodynamic bearing blades.
Therefore, it was decided to make an impeller suspended in the pump, working without the hydrodynamic forces coming from the hydrodynamic bearing. The levitation effect of the new impeller will be obtained by using an all-magnetic bearing generating magnetic forces through the stationary bearing and dynamic forces generated by the RH ROT pump motors.

Materials and Methods

As part of the material fabrication work, a reactive thiol-methacrylate system was evaluated. The reaction is based on a multi-step radical mechanism leading to high monomer conversion and excellent thermo-mechanical properties. The investigated and optimised resin system was evaluated in a 3D printing process in collaboration with Lithoz. It was found that the conversion of monomer functional groups in 3D printed parts was significantly lower than the conversion that could be achieved in thin model films. This behaviour can be explained by the lower intensity of light used in the digital light projection (DLP) printing process. Since methacrylates have significant cytotoxic potential, the amount of residual methacrylates in the printed parts should be reduced to the lowest possible level. To increase the conversion of functional groups, irradiation of printed parts at 80°C was investigated. This treatment significantly increases the conversion of methacrylates to 91% using 1.6 wt% Irg784 as photoinitiator. Furthermore, the effect of the choice and concentration of photoinitiator (PI) in the resin system on methacrylate conversion was also investigated. In addition to Irg784, the biocompatibility of the germanium PI Ivocerin was also investigated. A concentration of 2.4 wt.%. Irg784 leads to 86 % methacrylate conversion immediately after printing, which increased to 95 % after exposure (at 80°C). In a further experiment, the effect of post-curing on thermo-mechanical properties was investigated using dynamic mechanical analysis. Microstructure investigations were performed on two selected materials from the group containing carbon nanotubes. Cell viability was assessed after 24 h incubation using confocal microscopy. FDA (fluorescein tetraacetate) and PI (propidium iodide) were used in cell staining. Lipophilic FDA penetrates the intact cell membrane in a living and metabolically active environment.

Results and Discussion

It was found that additional irradiation at elevated temperatures leads to a significant increase in the glass transition temperature (51°C before and 66°C after curing. In order to increase the thermo-mechanical properties of the developed photopolymer, the addition of carbon nanotubes (CNTs) was planned. It should be noted that the addition of CNTs leads to a significant increase in resin viscosity. In order to overcome this limitation, the addition of thermal radical primers was evaluated. These compounds lead to the formation of radicals by thermally induced decomposition reactions. Based on the results obtained, there were significant differences in the density of nanotubes located in the materials. The density of nanotubes observed in the so-called bright field (BF) was very low. High-resolution analysis and inverse Fourier transform confirmed the presence of carbon nanotubes. Their effect on the material properties was evaluated in terms of cytotoxicity. The results of the direct cytotoxicity tests are shown in FIG. 1.

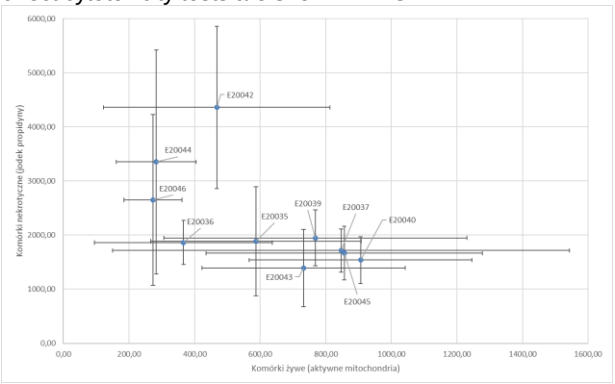

FIG. 1. Cytotoxicity analysis of the photosensitive materials.

Conclusions

The use of light-curing materials has no toxic effects on the surrounding tissues. The use of carbon nanotubes has a beneficial effect on reducing cytotoxic properties, but from a mechanical point of view, they reduce the ductility of the material.

Acknowledgments

Project supported by:
The Polish National Centre of Research and Development (Grant no. M-ERA.NET/2014/01/2016, “Nonthrombogenic metal–polymer composites with adaptable micro and macro flexibility for the next generation heart valves in artificial heart devices”).

POLYHYDROXYALKANOATE BIOPOLYMERS FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 29]

Introduction

Biopolymers are promising materials for tissue engineering [1]. One of such materials is a group of polyesters synthetised by microorganisms, namely polyhydroxyalkanoates (PHA). These biomaterials are completely biocompatible with mammalian tissues, which makes them appropriate for tissue engineering purposes. Depending on the (*R*)-3-hydroxyacid that constituents the polymer chain, the PHAs tend to vary in its physicochemical and mechanical properties (i.e., brittle, hard to elastic), offering a wide range of potential applications. Thanks to the broad features, these polyesters can be formed into purpose-specific materials through commonly used procedures. Moreover, PHAs can serve as a platform for controlled drug release which occurs locally only in the site of an implantation. Here, we present a complete route to obtainment and characterisation of two types of PHAs used for construction of wound patches.

Materials and Methods

Material synthesis Through microbial fermentation two PHA polymers were synthetised in 5L fermenters. *Z. denitrificans* converted glycerol to polyhydroxybutyrate (PHB), whereas *P. putida* converted octanoic acid to polyhydroxyoctanoate (PHO). PHAs were extracted form dried biomass, purified over charcoal, precipitated in methanol and resuspended in $CHCl_3$. Three methods were used to fabricate wound patches: electrospinning, wet-spinning and solvent casting-porogen (NaCl) leaching to water (SCPL). The materials were characterised by SEM or μ-CT. PHO was also modified with PHO-diclofenac-oligomers obtained by p-toluenosulphonic acid mediated synthesis [2]. *Biological characterisation* Mouse fibroblast cells were used for primary assessment of PHO-derived materials. Firstly, the cytoskeleton structures (actin, microtubule, vimentin, nuclei) were stained and observed using confocal microscopy in order to assess cell attachment and material impact on the cell morphology [3,4]. Further, migration studies were conducted, which were followed by *in vitro* wound healing assays [4]. Lastly, the SCPL patches (non and modified with diclofenac) were used in mouse *in vivo* model wound healing assay. Mice were sampled at weekly intervals (7, 14 days) and healing progress was assessed by three methods: immunohistochemically (IHC), by assessing the genome at the mRNA level by Real Time PCR (qPCR) and testing the interleukin level (IL) by enzyme immunoassay - ELISA (E).

Results and Discussion

Fermentation processes led to accumulation of two types of polymers. PHB was accumulated within 48 hours, biomass reached 96 g L^{-1} with 28% polymer content; whereas PHO process lasted for 30 hours and system accumulated 122 g L^{-1} of bacteria of which 71% was polymer. The PHAs were used to create three distinct structures (FIG. 1).

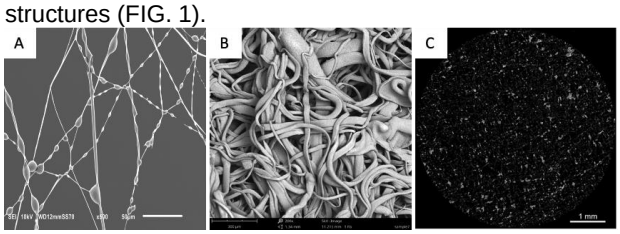

FIG. 1. Wound patches materials obtained by electrospinning PHO (A), wet spinning PHB (B) and SCPL method (C).

PHO was chosen for further studies. At first cellular morphology was observed of cells grown on flat PHO films. There were much more cytoskeletal bifurcations on the polymer than on glass, which suggests that PHO favours cell attachment. Next, cell migration studies revealed non-directional movement across the PHO surface, further confirming the biocompatibility of the PHA polymer. The *in vitro* wound assay allowed to conclude that PHO favours artificial wound closure, which is faster when compared to a polylactic acid control. Lastly, the synthetised PHO-diclofenac-oligomers were blended with PHO polymer, and its cytotoxicity was assessed. Based on the above preliminary studies two types of SCPL sponges were prepared: PHO-based and modified PHO with oligomers (100 μg/1g), which were tested in mouse model for wound healing capabilities. Application of either patch allowed for partial wound closure with material integration into the wound. Cells were migrating to the patches creating new blood vessels. There was low pro-inflammatory cytokine concentration and $CD68^+$ macrophages level, thus leading to the conclusion that materials are beneficial for the process of wound healing *in vivo*.

Conclusions

Fermentation process allows to obtain a wide range of PHA polymers, which can serve as substates for cell attachment and growth. PHAs can be easily turned into biomaterials with desired properties by commonly used methods (i.e. electrospinning, wet spinning or SCPL). On the example of PHO, it was proved that it is a promising material for wound healing process both by *in vitro* and *in vivo* studies. Further, PHO, being chemically pliable, can serve as a platform for local drug release.

Acknowledgments

Research funded by and the National Center for Research and Development, Poland, grant Techmatstrateg no. TECHMATSTRATEG2/407507/1/ NCBR/2019 and LIDER/27/0090/L-7/15/NCBR/2016.

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MODIFICATION OF THE SURFACE OF SPHERICAL ALUMINOSILICATES WITH GRAPHENE OXIDE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 30]

Introduction

The research is aimed at producing a new type of filler for composites based on spherical aluminosilicates. The aluminosilicate microspheres were subjected to the silanization process (APTES) and then doped with graphene oxide.

Materials and Methods

Spherical aluminosilicates (50 g) were etched in Caro acid (3:1 v/v), then the silanization process was propanol with APTES (10:1 v/v) for 12 h. The samples were filtered and dried at 80°C/24 h. Graphene oxide (25 g) was dispersed in 250 ml of HNO_3/H_2SO_4 mixture (3:1 v/v) for 30 min using ultrasound. The mixture was heated to 50°C for 30 min with constant stirring, the mixture was allowed to cool to room temperature. Before filtration, the mixture was neutralized to pH 7 in order to remove acid residues. Then the microspheres were filtered and dried in 100°C/24 h. The samples were calcined at 350°C, 450°C and 550°C/1,5 h in air [1,2].
Thermogravimetric analysis (TGA) with a heating rate of 10ºC/min (40ºC-1000ºC), scanning electron microscopy analysis (SEM-EDS), X-ray diffraction (XRD) and (FTIR) spectroscopy were used for the tests.

Results and Discussion

In this study, the aluminosilicate microspheres were surface modified in order to develop the surface by using the silanization process and doping with graphene oxide.
FTIR analysis showed -COOH and -OH groups, additionally nitrogen groups were detected, which may improve the chemical connection with the matrix of the composite. The shown groups may become a drug carrier in the further modification of the composite. X-ray analysis revealed peaks typical of cenospheres containing mainly aluminum silicate phases such as mullite and silimanite and other smaller phases such as quartz. The analysis showed several characteristic peaks related to the occurrence of disordered carbon structures. The structure of the microspheres in the SEM study showed surface development due to the use of the etching process and the silanization process.

Conclusions

The conducted research proved that the functionalisation of the surface of spherical aluminosilicate microspheres was right. Composites with the use of the produced filler may show lower density without losing mechanical properties, in addition, the use of graphene oxide as a drug carrier will help in the prevention of inflammation.

Acknowledgments

The project was funded by the Department of Biomaterials and Medical Devices Engineering, Faculty of Biomedical Engineering, Silesian University of Technology, Zabrze, Poland. The work is the result of the research Project no. 07/020/BKM_21/0070, BKM-602/RIB2/2021.

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PHYSICAL PROPERTIES OF PLGA BIODEGRADABLE POLYMER COATINGS CONTAINING HYDROXYAPATITE ON Ti6Al7Nb SUBSTRATE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 31]

Introduction

Titanium alloys are now one of the most widely used metallic biomaterials in medicine, especially as implants for osteosynthesis. Low density, high corrosion resistance and good biocompatibility in environmental of tissue and body fluids are properties which determine their usefulness [1]. However, after many years of research, was proved that they are not biologically inert, but may cause allergies and other adverse reaction [2]. For this reason and to improve biocompatibility, bioactivity and corrosion resistance, the use of a titanium alloy requires proper surface treatment [3,4]. One of the available modification methods may be the use of biodegradable polymer coatings, which, apart from improving biocompatibility by limiting the penetration of metal ions into the tissue environment, can be also a matrix for the release of the mineral and active substances. Released substances may have a beneficial effect on the bone tissue healing process and also reduce the need for systemic drug therapy [5]. Moreover, the degradation of the polymer will not deteriorate the mechanical properties of the implant, since the stability is ensured by the metal substrate [6]. Due to the frictional forces occurring in the implant-bone system, the adhesion and tribological properties of the coating are significant.
The aim of the study was to determine the properties of biodegradable polymer coating PLGA containing hydroxyapatite.

Materials and Methods

Ti6Al7Nb alloy samples were taken from a rod of 25 mm in diameter. The surface was subjected to modification, which included mechanical grinding, sandblasting and anodic oxidation. The anodic oxidation was performed in a bath containing phosphoric and sulfuric acid at 97 V at room temperature for 2 minutes. Polymer coating based on poly(D,L-lactide-coglycolide) PLGA(85/15) was synthesized in bulk by the ring opening polymerization of glycolide (Purac) and D,L-lactide (Purac) (first, at 130°C for 24 hours and next, at 120°C for 48 hours at argon atmosphere using Zirconium (IV) acetylacetonate ($Zr(acac)_4$) (Aldrich) as a non-toxic initiator. Solution of 1% PLGA in CH_2Cl_2 was enriched with 5%, 10%, 15% or 20% synthetic hydroxyapatite powder (<60 nm particle size) (Sigma Aldrich).
The substrate was coating by ultrasonic spray system ExactaCoat (Sono-Tek) with Impact nozzle. The coating process was carried out with ultrasound frequency 60 kHz and power 1.5 W. Flow rate of solution was 1 cm^3/min and speed of nozzle motion 5 mm/s. Coatings were composed of 5, 10 or 15 layers.
The scope of the research included microscopic observation of surface, wettability, adhesion and tribological tests.
The observations of the samples' surface were analyzed with a Zeiss Stereo Discovery V8 stereoscopic microscope (Zeiss) with a MC5s digital camera.
Wettability measurements were performed with distilled water and diiodomethane using a drop of liquid with a volume of 1.5 mm^3 The measurements were performed by applying the SURFTENS UNIVERSAL optical goniometer (OEG) and computer software Surftens 4.5 for. Tests were carried out at room temperature in 60 seconds with a sampling rate of 1 Hz.
Tests of adhesion of the polymer coatings to the Ti6Al7Nb substrate were carried out using the scratch test method, using an open platform equipped with a CSM Micro-Combi Tester. The tests were carried out with increasing loading force from 0,03 N to 30 N, loading speed 10 N/min, table speed 1 mm/min and crack length 3 mm.
Tribological tests were performed with Tribometr Pin-On-Disc (Anton Paar). Based on research from the literature [7] following parameters were accepted: friction rate 0,1 m/s and friction radius 8 mm.

Results and Discussion

The physical properties of PLGA polymer coatings containing various amounts of HAp (5, 10, 15, 20%) were determined using the wetting angle and surface free energy, the coating friction coefficient and its abrasion, as well as adhesion to the metal substrate. The coatings before and after the tests were observed with stereoscopic microscope.

Conclusions

The physical properties of biodegradable PLGA polymer coatings enriched with hydroxyapatite depend on the content of hydroxyapatite and the thickness of the coating.

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LIPID-POLYMER NANOCARRIERS FOR CARTILAGE REGENERATION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 32]

Introduction

Musculoskeletal ailments caused by cartilage damage are common, and thanks to more and more modern diagnostic methods, more and more often recognized. Moreover, cartilage diseases progress with age and are a consequence of injuries, becoming a dominant problem in orthopedic surgery. The conducted research aims to produce conjugates for cartilage regeneration based on chondrogenic differentiation of mesenchymal stem cells isolated from Wharton's jelly (hUC-MSC).

Materials and Methods

Kartogenin (KGN) – an active substance – was encapsulated in the liposomes. The carriers were covered with modified hyaluronic acid (Hy) or chondroitin sulfate (Ch) in order to obtain better stability of the systems. Ch was modified by octadecyl groups at two different substitution degrees, 15% and 30% (ChC18_15 and ChC18_30). Hy was modified by dodecyl or octadecyl groups at a similar substitution degree of 7% (HyC12 and HyC18). The physicochemical analysis of the obtained systems was carried out by dynamic light scattering, zeta potential, and fluorescence measurements. The thermotropic behavior of lipid membranes was studied using a Nano DSC calorimeter (TA Instruments). HUC-MSC morphology imaging after incubation with the prepared formulations and metabolic toxicity test were performed. Additionally, these systems have been tested to differentiate stem cells into chondrocytes using real-time PCR (rt-PCR).

Results and Discussion

Hy and Ch substitution by alkyl domains were confirmed by XPS spectra. KGN was successfully incorporated into the lipid bilayer. Composed formulations were stabilized by covering their surfaces with Hy and Ch derivatives. The changes in thermograms for an aqueous dispersion of DPPC confirmed the incorporation of polymers' hydrophobic domains into the lipid bilayer. Moreover, an increase in the liposome size and decrease in the ζ-potential values confirmed the presence of polymers on liposomes surfaces (FIG. 1). Despite both systems: modified GAGs and liposomes with KGN have high negative values, they interact with each other because of the hydrophobic effect. According to cytotoxicity results (MTT assay), all polymers used in lipid formulations were significantly non-toxic than pure polymers. This may be that the hydrophobic anchors are hidden in the lipide bilayer and their exposure to the cell surface is minimized. The selected genes expression was analyzed by real-time PCR. FIG. 2 shows only a couple of formulations. As can be seen, a lower dosage of liposomes with KGN that HyC18 coats give the best differentiation results. All systems have induced Col1A1 and SOX9 gene expression compared to untreated cells.

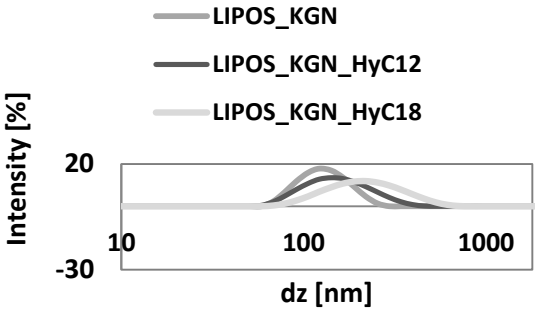

FIG. 1. DLS size distribution profiles of bare liposomes with KGN and coated with HyC12 or HyC18.

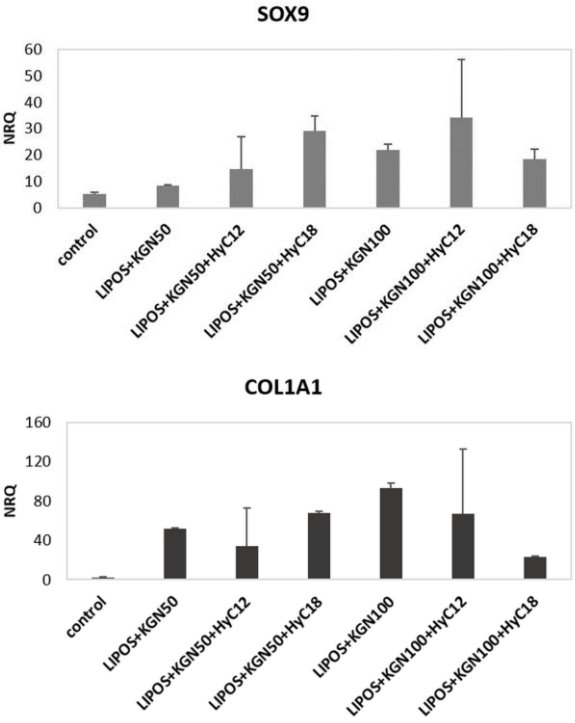

FIG. 2. Cartilage markers expression after stimulation by selected formulations, compared to control (untreated hUC-MSC).

Conclusions

Composed hybrid lipid-polymer formulations are stable vesicles as carriers of KGN. The resulting systems are promising conjugates for the regeneration of cartilage tissue.

Acknowledgments

This work was supported by the Polish National Science Centre, project no. 2016/21/D/ST5/01636.

MODERN POROUS POLYMER AND CARBON MATERIALS BASED ON POLYURETHANES FOR APPLICATIONS IN MEDICAL DIAGNOSTICS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 33]

Introduction

The analysis of biological samples dates back to the age of Hippocrates, who described halitosis (fetor oris) and hepatic stink (fetor hepaticus) in a treatise on the smell of breath and disease [1]. The potential for using the breath as a diagnostic sample has grown significantly with the advent of modern chemistry, materials, and biomedical engineering. In 1784 Antoine Lavoisier proved that the human body consumes oxygen and produces carbon dioxide, and six years later, he published a scientific article, "Experiments on animal respiration and changes occurring when air passes through the lungs" [2]. Based on Lavoisier's work almost 100 years later, in 1874, Francis Anstie observed that small amounts of alcohol were excreted in the exhaled air [3]. In 1897, Nebalthau showed that people with diabetes exhale acetone [4]. More systematic research and breathing tests did not appear until around 1927 when Bogen [5] and McNalley [6] developed alcohol tests, which initiated the development of the first device for testing sobriety in drivers developed by Harger in 1931 and patented in 1936 [7]. The modern era of respiratory phase testing began in 1971 when Pauling analyzed volatile organic compounds (VOCs) in the exhaled air that had been condensed in a chilled stainless steel tube. He found that normal human breathing contains over 250 different VOCs [8]. Since then, the area of respiratory phase analysis has attracted the attention of scientists as well as large interested physicians.

Materials and Methods

In this study, the activities related to the process of producing carbon foams (synthesis, carbonization) were described and their extensive characterization was made using the following research techniques: light microscopy, scanning electron microscopy (SEM), infrared spectroscopy (FT-IR), computer microtomography (μCT) and gas chromatography linked to a mass detector (GC-MS).
The results of the research were used to select foams with the best sorption properties, with a focus on the possibility of their further application for diagnostic tests of the respiratory phase.

Results and Discussion

The conducted research made it possible to identify porous materials with the best properties. Depending on the used substrates, their proportions and catalysts used, porous materials were obtained that differed from each other in terms of physical and mechanical properties. It was also found high homogeneity and chemical purity as well as no release of own volatile compounds, which could have an impact on the obtained results of the respiratory phase analysis. An additional advantage is the ability to manipulate the shape and size of the pores in the synthesis process.

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. The proposed method of respiratory phase analysis based on the use of synthesized porous polymeric materials is non-invasive and easy to carry out. Also, the method of obtaining material for research related to desorbing the respiratory phase from the foam is not difficult and is much simpler than in the techniques used so far.

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EFFECT OF Ta_2O_5 LAYER ON THE ANTIBACTERIAL AND PHYSICOCHEMICAL PROPERTIES OF NiTi ALLOY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 34]

Introduction

Shape memory alloys are widely used for implants such as plates for osteosynthesis, orthodontic wires or self-expanding stents, because of their biocompatibility and self passivating properties. However, they require additional surface improvement to prevent harmful nickel migration. This can be achieved through different methods, such as layering [1]. A promising method for applying thin films is the Atomic layer deposition (ALD), which allows the fabrication of films with homogeneous, controlled thickness [2,3]. Some of NiTi biomedical devices implanted by minimally invasive methods should be clearly visible under X-ray light. Nickel-titanium alloy does not meet this requirement, despite its several advantages. An additional challenge is to develop a layer that is resistant to susceptibility to deformation during the implantation procedure. Therefore, a tantalum oxide Ta_2O_5 layer applied by ALD method was proposed [4,5]. In addition, the effect of the resulting layer on bacterial biofilm formation was investigated due to complications that may arise from the spread of infection. A biofilm is a cluster of microorganisms consisting of bacteria and fungi. Approximately 60-70% of microorganisms settling on the implant surface are related to existing nosocomial infections [6].

Materials and Methods

The test material was superelastic NiTi (55,6% Ni) for implants with an austenite finish temperature A_f = 34°C. Disks with a diameter of 14 mm were cut from 0.8 mm thick sheet metal. The alloy chemical composition fulfills the requirements of ASTM 2063-18. Next, the specimens were electropolished in 3.5-mol H_2SO_4 solution at 0.75 A/cm^2 and 18 V for 5 min [7,8]. After this treatment, all of them were cleaned for 10 min in an ultrasonic bath in 96% ethanol. Then, a part of them were passivated to produce an additional intermediate surface layer between the NiTi alloy and the tantalum oxide layer (TABLE 1).

TABLE 1. NiTi substrate preparation alternatives.

No.	Passivation method
I	initial state - electropolished NiTi
II	digestion with Kroll's reagent and boiling for 2 hours in 30% H_2O_2

The Ta_2O_5 layer was obtained using $Ta(OC_2H_5)_5$ and water as precursors. The process was carried out in 400 cycles and 300°C [9]. At the end, such material was sterilized in an autoclave, to remove microorganisms. The adhesion of the layer to the substrate, wettability, surface tension and pitting corrosion resistance were investigated. Moreover, bacterial strains were used for microbiological studies.

Results and Discussion

The corrosion resistance results indicated that the material could be used for implants (FIG. 1). Before coating, each of the variants had very good corrosion resistance. After the layer was applied, the polarization resistance of all variants increased, but only in the case of perhydrol there was no breakthrough of the passive layer.

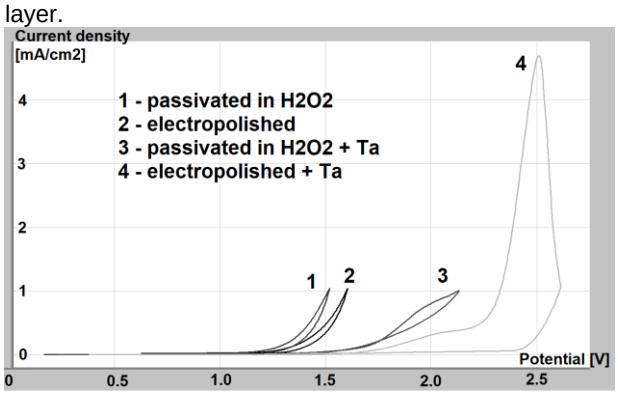

FIG. 1. The corrosion resistance of NiTi uncoated and coated by Ta_2O_5 layer.

Adhesion test data (TABLE 2) showed differences depending on the surface preparation.

TABLE 2. Results of Ta_2O_5 layer adhesion tests.

No.	Coating damage	F [N]
I	Delamination Lc2	20,73
	Complete break Lc3	27,27
II	Delamination Lc2	11,28
	Complete break Lc3	13,41

Surface wettability study concluded that the ALD coating reduces the wetting angle, making the surface more hydrophilic, which may have a negative effect on hemocompatibility. However, further biological studies are required. Also, the various effects of the layer on bacterial adhesion were confirmed.

Conclusions

The effect of surface preparation of NiTi alloy for implant application on the physicochemical properties of tantalum oxide layer applied by ALD method was investigated during this study. Differences in bacterial adhesion to NiTi alloy with and without Ta layer were observed.

Acknowledgments

The work is the result of Research Project No. 07/020/BKM_21/0069.

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MULTIFUNCTIONAL BIOMATERIALS BASED ON NANOSTRUCTURED ANODIC TITANIUM DIOXIDE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 35]

Introduction

An increasing number of bone implants determines an increased interest in developing novel implantable materials and improvement in currently used ones. One of the most commonly applied biomaterials in orthopedics and dentistry is titanium and its alloys. It is mainly due to their favorable properties, such as corrosion resistance, good biocompatibility, and decent mechanical properties [1]. However, the osseointegration process between the implant and the surrounding tissue, which happens through the natural titanium oxide (TiO_2), is inefficient. That is why scientists are applying various surface modifications, among which electrochemical oxidation (anodization) of metals seems promising [2]. The method is cost-effective and straightforward, and what is more important, it allows for synthesizing nanostructures with precisely controlled morphology. Such morphology, comprised of vertically aligned nanocontainers, not only provides a biocompatible surface for cells growth [3] but also enables for the incorporation of different compounds, e.g., drugs or antibacterial agents [4], growth factors, which ensures that such materials possess multifunctional properties [5,6].
Anodization was recently applied for three-dimensional substrates (3D –printed), which opens a wide range of new possibilities and challenges for synthesizing complex biocompatible biomaterials [7,8].

Materials and Methods

Anodic titanium oxide (ATO) layers on 2D Ti foil, Ti-based alloys (Ti13Nb13Zr and Ti15Mo), and 3D Ti substrates were prepared via the anodization process conducted in the ethylene glycol-based electrolyte with the addition of NH_4F and H_2O, under a constant voltage in the range between 40 and 100 V at 20°C. The obtained oxide layers were characterized by their physicochemical properties by using different methods, e.g., SEM, EDS, XRD, XPS, and contact angle measurements. 2D and 3D Ti samples were used as drug delivery systems, and their detailed characterization as drug carriers was provided. Some of the synthesized materials were also modified with Ag nanoparticles in terms of their antimicrobial properties. Finally, the biocompatibility of such layers was also examined.

Results and Discussion

Different anodization procedure was applied depending on the used substrate (Ti foil/cube or Ti-based alloy), which resulted in different morphologies of the obtained nanostructured oxides. Received ATO layers differed in the pore diameter and oxide thickness, which then impacted their properties, both physicochemical and biological. All anodized samples showed better wettability when compared with bulk substrates. Corrosion parameters differed for all of the examined materials. The surface properties of the examined biomaterials were compared with their biocompatibility. What is more, the successful deposition of silver nanoparticles on ATO layers was performed. Such modified materials were characterized by slightly better antibacterial activity towards Gram-positive and Gram-negative bacteria. ATO layers also presented enhanced osteoblast-like cells growth when compared to bare substrates. Finally, 2D and 3D samples modified with titanium dioxide were used as drug delivery systems for ibuprofen and/or gentamicin. It was proved that drugs could be successfully loaded and released from such materials. What is more, a kinetic model was fitted to the release profiles, describing the nature of the process in detail.

Conclusions

To sum up, it was shown that electrochemical oxidation of titanium-based materials leads to the efficient modification of the metallic surface of various initial geometries. Such biomaterials enhance cells adhesion and proliferation but also may be used as drug carriers. Combined with chemical changes with antibacterial agents, they gain also new properties, and thus, may be considered as multifunctional biomaterials.

Acknowledgments

Magdalena Jarosz would like to acknowledge financial support from the National Science Centre (no. UMO-2017/25/B/ST8/01599).

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WHAT NEW EU REGULATION MEANS FOR NANOMATERIALS DEVELOPMENT DEDICATED FOR MEDICAL DEVICES UTILISATION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 36]

Introduction

New Medical Devices Regulation (MDR) applies from May 26, 2021 [1]. The new legal regulations point out that in the design and manufacture of medical devices, special care should be taken when using nanoparticles for which there is a high or medium potential for internal exposure. Such devices should be subject to the most stringent conformity assessment procedures.

Nanomaterials have been widely used in medicine and pharmaceuticals because of their specific mechanical, optical and electrical behaviours. They are applied for the detection of biological molecules, imaging of diseased tissues and innovative therapeutics [2].

The detailed review of defined requirements was carried out, in the aspect of research, properties, and manufacturing of biomaterials for use in medical devices, with particular emphasis on the safety of nanomaterials.

Materials and Methods

According to the definition of a medical device, the material itself may be a medical device, e.g. tissue adhesives, tissue prosthesis: vascular grafts, tissue patch, bone restoration, as well as cellular vehicles, etc. The following new aspects of regulations, having a huge impact of nanomaterials development, were analysed:

- definition of a nanomaterial, which appears in the regulations for the first time,
- special classification rule implemented for medical devices incorporating or consisting of nanomaterial,
- risks of particles which are or can be released into the patient's or user's body,
- general safety and performance requirements such as chemical, physical and biological properties,
- new standards regarding material and nanomaterial biocompatibility assessment.

Results and Discussion

Nanomaterial, in the aspect of medical devices, means “a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate, and where for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm”. Moreover, fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm are also deemed to be nanomaterials. [1]

Due to the risk, all devices incorporating or consisting of nanomaterial are classified as class III, IIb or IIa depending on the internal exposure potential.

Special attention shall be given to nanomaterials during medical devices design and manufacturing, in such a way as to reduce as far as possible the risks linked to the size and the properties of particles which are or can be released into the patient's or user's body, unless they come into contact with intact skin only. The risk analysis according to EN-ISO 14971:2019 standard should be done in the beginning of the medical device design process.

Devices shall be designed and manufactured in such a way as to ensure the general safety and performance requirements are fulfilled. The following biomaterial properties should be considered:

- the choice of materials and substances used in medical device, particularly as regards toxicity as well as all others biocompatibility aspects, in accordance to ISO 10993 standard;
- the compatibility between the materials and substances used with biological tissues, cells as well as body fluids, taking account of the intended purpose of the device and, absorption, distribution, metabolism and excretion where relevant;
- the impact of processes on material properties (manufacturing processes like mould injection, dipping, additive manufacturing, etc; surface engineering; cleaning, disinfection and sterilisation; etc.);
- the mechanical properties of the materials used, reflecting, where appropriate, matters such as strength, ductility, fracture resistance, wear resistance, and fatigue resistance, regarding materials after all technological processes applied in the medical device manufacturing;
- surface properties (topography, roughness, wettability etc.).

ISO/TR 10993-22:2017 standard provides a general framework and highlights important aspects which need to be considered when assessing the safety of medical devices composed of, containing and/or generating nano-objects.

For medical device’s producer, the confirmation that the device meets any defined chemical and/or physical specifications is crucial and should be supported by technical data sheets delivered by biomaterial/ nanomaterial manufacturer.

Safely utilisation of medical devices with the materials and substances, including gases, with which the devices enter into contact during their intended use, should be also evaluated, especially, if the device is intended to administer medicinal products.

Devices shall be designed and manufactured in such a way as to reduce as far as possible the risks posed by substances or particles, including wear debris, degradation products and processing residues, that may be released from the device.

The risks associated with the use of the device when it comes into contact with materials, liquids, and substances, including gases, to which it is exposed during normal conditions of use, should be also taken into account.

Conclusions

There is scientific uncertainty about the risks and benefits of nanomaterials used for medical devices. In order to ensure a high level of health protection, special attention risk management process, including risk analysis should be implemented in the very beginning of nanomaterials development in the aspect of medical devices utilisation. In the light of technical and scientific progress the definition of nanomaterial can be changed. If the new nanomaterials development process is performed in accordance with all the regulation requirements, the long time to the market of medical device composed of, containing or generating nanomaterials is reduced. For new nanomaterials the more biocompatibility properties of raw material is confirmed by the material developer, the biggest commercial potential is available in the aspect of medical device market.

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SURFACE FUNCTIONALIZATION OF OPEN-CELL STRUCTURES WITH DLC COATINGS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 37]

Introduction

Porous structure is a universal term used to describe the size, distribution and morphology of pores of a material. Porous structures can be classified by porosity types (closed and open pores) and by the arrangement of elementary cells (stochastic and non-stochastic) [1,2].

In recent years, there has been increased interest in additive manufacturing porous structures for biomedical applications. In this direction, the usefulness of structures with the triply periodic minimal surfaces (TPMS) topology was investigated [3-5].

The architecture of the implant (pores size, shape, volume fraction and distribution) affects not only mechanical properties. It also has a significant impact on ensuring the correct integration of the implant into the biological environment through the process of osseointegration and neovascularization [6,7].

In fact, not only the mechanical properties and porous structure are important in the osseointegration process. Surface properties such as roughness, antytrombogenicity and the prevention of pathogens adhesion play an important role in in the process of bone osseointegration.

Materials and Methods

In this study, nTopology (nTopology GmbH, Germany) software was utilized to design the cylindrical mapped TPMS lattice structures with shell gyroid unit cells.

The samples were fabricated using a ORLAS CREATOR® selective laser melting (SLM) machine. from 316L austenitic stainless steel powder with an average particle size of 45±15 μm, which was produced in gas atomized process by Oerlikon Metco Inc., USA (MetcoAdd^{TM} 316L-A).

The surface of the samples was modified with the use of DLC coating.

Due to the complex open-cell structure of the samples, it is necessary to analyse the degree of surface coverage by the layer. Coating tests were included, inter alia, the study of morphology with the use of the digital optical microscopy, confocal microscopy, scanning electron microscopy with EDS analysis (SEM-EDS), crystallographic structure and chemical composition with the use of the X-ray diffraction (XRD) and scratch test and microhardness test. The biological examined the cytotoxicity and bactericidal properties of DLC modified metallic samples compared with and non-modified.

Results and Discussion

The preliminary results show the differences between the samples covered by DLC coatings in compared with non-modified in mechanical properties and surface morphology.

Conclusions

The DLC coatings onto metal samples obtained by selective laser melting (SLM) method create possibilities to manufacturing biocompatible biomaterial with controlled surface activity.

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BIODEGRADABLE IRON-BASED MATERIALS FOR CARDIAC PURPOSES — WHAT WAS DONE AND WHAT MORE CAN BE DONE?

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[ENGINEERING OF BIOMATERIALS 163 (2021) 38]

Introduction

Coronary artery disease (CAD) is characterised by narrowing of the blood vessels that supply oxygenated blood to cardiac muscles: it is responsible for around 20% of all deaths in developed countries [1]. In 1977, for the first time, an angioplasty was performed. This procedure, using a balloon inserted into a narrowed blood vessel and then inflated, enabled the vessel to be restored and prolonged the life of the first 38-year-old patient by 37 years [2]. Balloon angioplasty, however, was limited by unpredictable vessel dissection and recoil and by the high rate of restenosis. Therefore, the next revolution in the treatment of cardiovascular medicine was the introduction of stents, which resulted in both better early results and lower rates of restenosis. At the same time, there were limitations due to stent thrombosis and neointimal hyperplasia resulting in vasoconstriction. In 2007, Mani et al. formulated nine features that an ideal stent should have: (1) good expandability ratio; (2) ability to be crimped on the balloon catheter; (3) sufficient flexibility; (4) sufficient radial hoop strength and negligible recoil; (5) non-toxicity for tissues and all organisms; (6) high thromboresistivity; (7) absence of restenosis after implantation; (8) drug delivery capacity; and (9) adequate radiopacity/magnetic resonance imaging (MRI) compatibility [3]. In the meantime, the idea of biodegradable (or bioresorbable) stents arose. The biggest advantage of biodegradable stents (BDS) is that they disappear when they are no longer needed, which is about six months after the implantation. In this way, all late stent complications, like permanently diminished flow of covered side branches, bleeding problems associated with long term anticoagulation, permanent late fracture abnormal vasomotion and CT/MRI imaging artefacts are omitted. At the same time, BDS provides mechanical support analogous to bare-metal stents. It is also a better solution for still-growing children because it helps avoids a second intervention to remove the implant. This is why the list of features of an ideal stent should include a tenth feature: fully biodegradable.

Materials used to create bioresorbable stents

Two types of materials are used to create bioresorbable stents or scaffolds (BRS): polymers and metals. Initially, more attention was paid to polymers, and already in 1998, so over 20 years ago, a scaffold composed of high-molecular-weight poly-l-lactic acid (PLLA) monofilaments) was implanted per Igaki-Tamai into a human coronary artery [4]. The first report, where a total of 25 scaffolds were successfully implanted into 19 lesions of 15 patients, were described and published in 2000. Long-term (>10 years) studies in 50 patients showed that, after three years, no traces of the stent scaffold in the blood vessel could be detected [5]. The results were great, but the device failed to progress as it required a larger guide catheter for implantation than a metal stent, it needed a heated contrast, and it had the lack of a drug coating. However, the proposal to use PLLA in stents was not forgotten, and research is still ongoing. Other biodegradable polymers and copolymers used for research include: poly(ε-caprolactone) (PCL), poly(l-lactide-co-ε-caprolactone) (PLCL), phosphoryl choline (ChoP), etc. Despite very promising results, the polymers also have several disadvantages that limit their use. Compared to metals, polymers have lower values of Young's modulus (0.2–7.0 GPa) than those of metals (54–200 GPa), and generally, have poorer mechanical properties [6]. This makes the spacers in polymer stents thicker than in metal stents, which results in the impossibility of complete expansion as the balloon expands. Therefore, more and more research is being done to create a biodegradable metal stent. Metals degrade in the body through corrosion. Therefore, metals used in first-generation stents, such as stainless steel, nitinol or titanium, which has a high corrosion resistance factor, cannot be used as resorbable materials. From research conducted over the past 20 years, three main metals have emerged that could potentially form biodegradable cardiovascular implants: Fe, Mg and Zn [7-9]. Magnesium BDS are completely biocompatible and have good mechanical properties. However, magnesium has a high corrosion rate, which means it loses integrity when it is still needed. Moreover, it releases hydrogen during degradation that is harmful to cells. Zinc is characterised by good biocompatibility and a corrosion factor adequate to the desired lifetime of the stent. However, its mechanical properties are too weak. It is necessary to introduce modifications to improve the mechanical parameters and, at the same time, not affect the corrosion time. Iron has high strength, ductility, and formability, allowing stents with thinner constructions and struts or fabrication of special shapes, like foils or foams. Unfortunately, in comparison to Zn and Mg, iron has a corrosion rate so low that pure iron can hardly be called "biodegradable". But due to its biocompatibility and excellent mechanical properties, it is worth thinking about modifications that could accelerate corrosion.

In the presentation, the information about the iron properties, its biodegradability and its corrosion test, which can be carried out in immersion mode and during the electrochemical testing will be discussed. Biological properties of iron-based materials, in terms of tissue biocompatibility, cellular biocompatibility, hemocompatibility, and clinical biocompatibility are presented and discussed also. A critical look at the rate of degradation of systems obtained by several different synthesis methods, including: spark plasma sintering, vacuum induction melting, vacuum arc melting, electroforming, powder metallurgy and template-based synthesis of porous materials, as well as by the addition of another phase to the iron, will allow the reader to select methods which are still worth optimizing because they give hope for their use in biomedical applications, and those that they do not provide any chance of obtaining iron-based material as an optimally biodegradable system.

Acknowledgments

Authors would like to acknowledge National Science Centre for the financial support of research within PRELUDIUM BIS grant no. 2019/35/O/ST5/00405.

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INFLUENCE OF CRIMPING PROCESS ON RADIAL STRENGTH OF VASCULAR STENT MADE OF VARIOUS BIORESORBABLE POLYMERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 39]

Introduction
The World Health Organization estimated 17.9 million people died from Cardiovascular diseases in 2019, which is 32% of all deaths worldwide [1]. The scale of this problem affects the development of new technology in medicine cardiovascular area. Bioresorbable Scaffolds (BRS) are a promising medical tool for revascularization of a occlusive coronary artery disease. Basically, BRS are a scaffold for healing vassel which helps restoring vasomotive function and leaving no implant behind [2,3]. This advantage in comparison to metal vassculary stent is a driving force for developing bioresorbable stent technologies. Comperes metal and polymers stent production, the many differences could be found. Herein, we focused on crimping process of bioresorbable Poly(L-lactide)'s copolymers stent, on catheter which causes the significant change in radial strength whereas metal stent does not exhibit such phenomena [4].

Materials and Methods
The stents of Poly(L-lactide)'s (PLLA) copolymers and blends were manufactured using Micropowder 15 micro injection machine, made by Battenfeld. The radial force of stents was determined using radial force machine manufactured by Blockwise model RTU 124.

Results and Discussion
It was observed that final radial strength of opened biodegradable stent was lower after process of crimping on a balloon catheter. The changes in the value of radial strength were depended on Poly(L-lactide) contant in material and temperature of crimping. The observed changes are most likely due to the shape of the stent in which the bending point of the elbows enabling the stent to be packed to a diameter of 1.1 mm from 3mm or even 3.5mm diameter. During this process, deformation of the elbows(strut) undergoes more than 100%. As a result of such a deformation, the material may lose phase continuity which causes a decrease of material strength in span. In addition, PLLA is known for its high crystallization ability, which can affect the brittleness of the material during deformation. Therefore, stents made of materials with increased PLLA content lose the most radial strength after the crimping process.

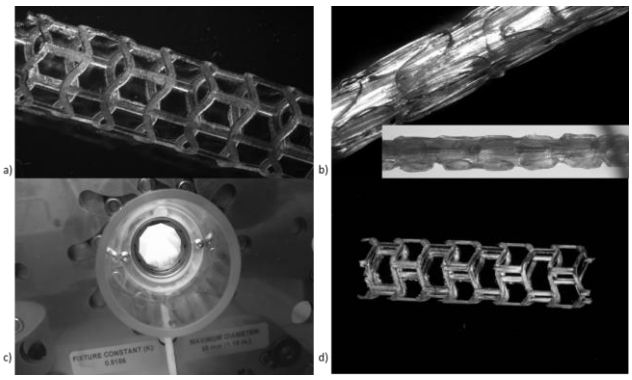

FIG. 1. Stent: a) before crimping process, b) crimped on balloon catheters, ready to implantation, c) crimper, d) opened after crimping (bright area-material tension)

Conclusions
The observed changes can be minimized by optimizing the shape of the stent, reducing the level of local deformation, in addition, it is important to adjust the material composition avoiding excessive amount of component with high crystallization tendency.

Acknowledgments
The research was carried out as part of project of The National Centre for Research and Development No. POIR.04.01.02-00-0105/17-00 "The technology for developing and obtaining the next generation of vascular stents through microinjection".

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FUNCTIONALIZATION OF CARBON BIOMATERIALS SURFACES: FUNCTIONAL GROUPS AND NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 40]

Introduction

The application of nanomaterials in biomedicine is one of the most demanding challenges in the newly emerging bionanotechnology field. The main advantage of nanoparticles (NPs) is the possibility to control their physicochemical properties by modifying their bulk and surface composition, size, and morphology, which can be further optimized through various functionalities (e.g., peptides, antibodies, genes, drugs). Because of their biocompatibility and unique properties, such as surface plasmon resonance or superparamagnetism in some cases, NPs attract broad interest for applications in cancer therapy, pharmacology, advanced diagnostics, antiseptics, and treatment of bacterial infections. The last two applications are of particular interest for studies aiming at targeting pathogenic bacteria with alternatives to antibiotics. One of the approaches used in this context involves the use of NPs as biocidal agents. Among nanoparticles exhibiting antibacterial activity, previous studies have mainly focused on various metals and metal oxides, owing to specific features including electronic, magnetic, and optical properties. Although many metals exhibit antimicrobial properties, the NPs investigated in this context are based mainly on silver, copper, zinc, gallium, and gold. Metal oxide nanoparticles such as ZnO, CuO, Fe_3O_4, TiO_2, VO_2, MgO and NiO are also considered as potential antimicrobial agents.

In this study, we investigated the microbiological response of graphenic surfaces functionalized with oxygen surface groups and nanoparticles of Ag and Au. The obtained results provide fundamental insights which may be used for designing and developing smart materials with functional surfaces exhibiting bacteria-attracting and/or bacteria-killing properties.

Materials and Methods

Citrate-capped metallic nanoparticles were synthesized following the protocols described elsewhere [1]. Oxygen plasma was applied to generate –OH and –COOH surface groups on graphenic materials. A sonochemical method was employed for the deposition of the synthesized nanoparticles on model graphenic sheets (10 min, 30 W cm^{-2}). The obtained materials were characterized with the use of SEM, TEM, XPS, SIMS, work function measurements. Microbiological tests with the use of Gram-positive (*S. aureus*, *S. epidermidis*) and Gram-negative (*P. aeruginosa*, *E. coli*) were performed.

Results and Discussion

The main idea of the work is presented schematically in FIG. 1. Upon oxygen plasma treatment the graphenic surfaces were effectively functionalized: increase of hydrophilicity (decrease in water contact angle) and electrodonor properties (increase in work function). These changes in surface properties were strongly reflected in bacteria adhesion. The synthesized nanoparticles were sonochemically deposited over graphenic surfaces and their effects on bacteria adhesion were also evaluated. The nanoparticle sizes evaluated by TEM were 26 and 24 nm for AgNPs and AuNPs, respectively. It was found that surface functionalizations with AuNPs and AgNPs have opposite effects on bacterial attachment for all the investigated bacterial strains.

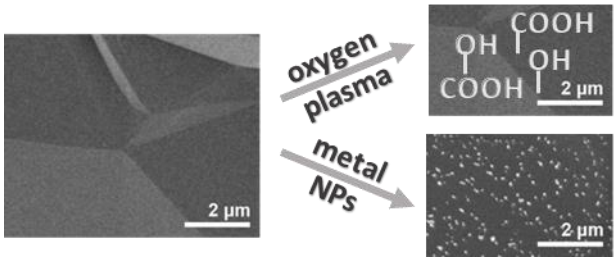

FIG. 1. SEM micrographs of graphenic sheet before (large image), after oxygen plasma treatment (upper insert) and decoration with gold nanoparticles (lower insert).

Gold NPs were found to be more effective in promoting bacterial adhesion (reaching a ~100% increase for Gram-positive bacteria after 1 h of incubation) than silver NPs (with a ~25% decrease). It was revealed that graphenic surfaces with co-deposited Ag and Au nanoparticles exhibited the combined effect of the double functionalization, i.e. increased bacterial adhesion (stimulated by AuNPs) and enhanced bactericidal effect (provided by AgNPs). SEM observations of bacteria morphology revealed the presence of many outer membrane vesicles. These structures are produced by microorganisms as a result of metabolic processes and participate in extracellular transport, bacterial biofilm formation, intercellular signaling; they are formed under the influence of stress factors such as AgNPs, Ag^+ ions, and reactive oxygen species. The latter was proposed as responsible for the observed antibacterial properties of the functionalized graphenic surfaces

Conclusions

The experimental correlations established in this study provide a background for the knowledge-based functionalization of graphenic materials via generation of oxygen functional groups and deposition of metal NPs. The observed effects were discussed in terms of surface–microorganisms interactions (attraction/repulsion and bacteriostatic/bactericidal effects) which can be dramatically change upon both kinds of modifications.

Acknowledgments

This study was funded by Polish National Science Centre project, award number 2020/37/B/ST5/03451

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SURFACE FUNCTIONALIZATION OF POLYURETHANE BIOMATERIALS: COMBINED EXPERIMENTAL AND MOLECULAR DYNAMICS SIMULATIONS APPROACH

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[ENGINEERING OF BIOMATERIALS 163 (2021) 41]

Introduction

Polyurethanes are a large family of polymers widely used in medical devices with one common characteristic of the presence of urethane linkages along the large molecular chains. Properties such as elastomeric and fatigue resistance are the basis of polyurethanes' success in biomedical science. Owing to their versatility of chemical composition, polyurethanes are now the highest-performing biomedical-grade elastomers. The availability of polyurethanes in several forms, i.e. of adhesives, coatings, sealants, rigid and flexible foams as well as textile fibres all of them in wide hardness range (from soft to super hard in Shore hardness scale) allow their use in pacemakers, catheters, vascular grafts, heart assist balloon pumps, artificial heart bladders and wound dressings [1].

The chemical modification strategies to prevent bacterial colonization have employed different types of bactericidal substances (e.g., metallic nanoparticles, peptides, antibiotics), among which antibiotics are most frequently used. Although the worldwide epidemic of antibiotic resistance is in danger of ending the common antibiotic therapy, there is still a lack of better drugs substitutes that can effectively prevent and cure infections. Antibiotic resistance cannot be restrained, but its development and spread can be significantly slowed down by curtailing a large amount of unnecessary antibiotic use in medical treatment. Therefore, implants surface functionalization to prevent biomaterial-centered infection (BCI) states an attractive alternative option to the typical prevention pathways. Therefore, understanding the factors affecting bacterial adhesion to surfaces is crucial for the design of novel and safe biomaterials.

The study aimed to optimize the plasma modification parameters of polyurethane surfaces with oxygen-containing surface groups. Such functionalization enhances the interactions between body fluid ions and the polymeric surfaces, observed experimentally as calcium phosphate formation, peptide (RGD) and protein (casein) adsorption. The experimental investigations were complemented with the use of molecular dynamics simulations which allow for an in-depth understanding of the interfacial processes.

Materials and Methods

Oxygen plasma modification. To modify the polymeric surfaces, oxygen plasma treatment was carried out using a Diener electronic Femto plasma system at 50 W and an oxygen partial pressure of 0.14 mbar. The varied parameter was the time of exposure to the plasma, which was in the range of 0.1−10 min.

Materials characterization. The samples were characterized with the use of spectroscopic (ATR-IR, XPS, SIMS, DSC) and microscopic (fluorescence microscopy, SEM) methods. The adsorption of peptides and proteins was followed by Quartz Microbalance.

Atomistic Molecular Dynamics Simulations. Fully atomistic molecular dynamics simulations were performed to investigate interactions between water molecules and several surfaces modelling polyurethane. In our model, the slab configuration was used with two model surfaces interacting with a slab of water [2].

Results and Discussion

Upon oxygen plasma treatment, the originally hydrophobic polyurethane surfaces (Θw=99º) turn into hydrophilic and a dramatic decrease of water contact angle value to Θw=0.1º (50W, 0.14 mbar, 6 min) was observed. As a consequence, the calculated values of Surface Free Energy (SFE) were changed accordingly. Initially, the SFE of unmodified polymer is 28.1 mJ/m^2 and consists mostly of the dispersive component. Modification with oxygen plasma results in the incorporation of oxygen-containing surface groups such as -OH, -CHO and -COOH, as identified with the use of XPS (FIG. 1A). The functional groups alter significantly the kinetics of RGD and casein adsorption, which is a good measure of biocompatibility of the modified polyurethane surfaces.

Moreover, the experimental measurements were complemented with theoretical studies using MD simulations. A representative image of the N atom distribution of the polyurethane chains in the simulation box is presented in FIG. 1B. The simulations allow determining which surface functional surface coverage is optimal in terms of biocompatibility and in a broader perspective allow to design functionalized implantable polymeric materials and their fine tuning.

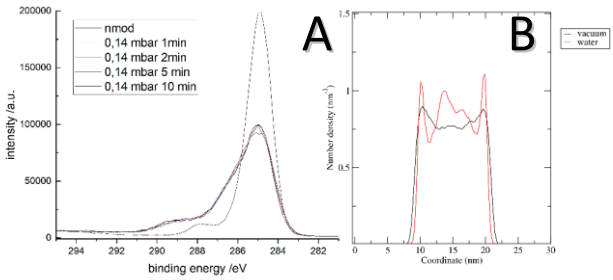

FIG. 1. Representative C1s XPS spectra of unmodified (nmod) and oxygen plasma modified polyurethane (A) accompanied by a representative image of polyurethane chains N atom distribution in the MD simulation box (B).

Conclusions

The study clearly shows that such a comprehensive experimental and theoretical approach allows for knowledge-based design and optimization of polymeric biomaterials interfaces, crucial in terms of biocompatibility and lowering the risk of BCI.

Acknowledgments

This study was financed by the Polish National Science Centre project awarded by decision number DEC-2019/35/D/ST5/03107.

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SONOCHEMICAL FORMATION OF BIOACTIVE NANOPARTICLES AND THEIR EMBEDMENT IN POLYURETHANE SURFACES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 42]

Introduction

Nanotechnology represents a dynamically developing section of the research and techno-economic sector with various application areas. Nanometric materials (especially nanoparticles) often exhibit properties dissimilar to their bulk equivalents because of the increase in the surface area, unsaturated sites, quantum effects, etc. As a result, fundamental studies for the generation, processing, characterization, and modelling of nanoparticles are carried out extensively. Nanoparticles and nanostructured materials are considered as a potential for revolutionizing medicine and create new classes of drugs and medical devices. One of the promising and efficient techniques for the synthesis of both inorganic and organic nanoparticles is sonochemistry. This approach is particularly interesting in terms of using it for nanoparticles of bioactive substances fabrication. Moreover, it provides a possibility to develop new, advanced materials which meet the demands of high-tech applications, e.g. in-site controlled drug delivery systems (DDS) [1-3].

The detailed understanding of the mechanism of nanoparticles formation via the sonochemical method is challenging. To investigate the impact of ultrasounds on the first steps of nanoparticles formation we coupled the experimental approach with the Molecular Dynamics simulations (MD).

Materials and Methods

Polyurethane (aromatic polyether polyurethane, American Polyfilm, Inc.) films were modified using oxygen plasma (FEMTO system, Diener Electronics). Fluorouracil and carbenicillin nanoparticles were formed and deposited on the oxygen plasma modified and unmodified polyurethane surfaces using a homogenizer (Sonics Vibracell CV18). The size of the sonochemically formed NPs was determined using the LM10 Nanosight instrument (Malvern Instruments Ltd) equipped with a sCMOS camera (Hamamatsu Photonics, Hamamatsu, Japan) and a 450 nm blue laser. Data were processed with NTA software version 3.1 Build 3.1.45. The developed system was thoroughly characterized in terms of particle size (NTA, TEM) and surface (ATR-IR).

The atomistic MD simulations were carried out in an NVT ensemble using GROMACS 5.1.x software and the parameters for bioactive molecule were taken from the Amber03 and for water form TIP3P force field.

Results and Discussion

The developed system, as well as nanoparticles, were thoroughly characterized by spectroscopic and microscopic methods. The sonochemical synthesis parameters were optimized to obtain nanoparticles of desired size, with the use of NTA set up. The adjusted parameters of the ultrasound generator resulted in nanoparticles up to 100 nm for fluorouracil and carbenicillin. As this method includes the hydrodynamic diameter, to directly measure nanoparticles size, TEM observations were conducted. The obtained nanoparticles were amorphous with a spherical shape (FIG. 1).

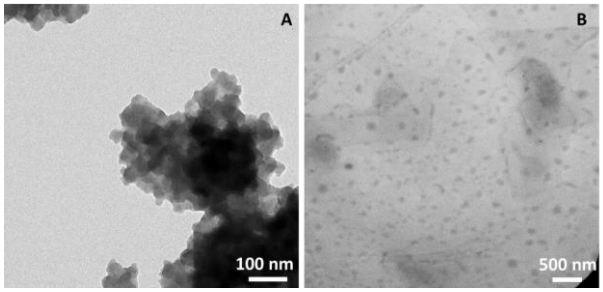

FIG. 1. TEM image of sonochemically created fluorouracil (A) and carbenicillin nanoparticles (B).

The impact of ultrasounds on the molecular structure of bioactive substances was also investigated with the use of ATR-IR spectroscopy. The therapeutic function of the polyurethane surfaces was obtained in one-step sonochemical process with the use of optimized parameters for fluorouracil and carbenicillin nanoparticles formation. The embedment of nanoparticles in polyurethane structure was then confirmed using IR spectroscopy. An apparent increase in the absorbance for a characteristic band (1640-1720 cm^{-1} and 1750-1800 cm^{-1}, for fluorouracil and carbenicillin, respectively) was observed for drug–containing samples when compared to the parent polyurethane, indicating the presence of the drug.

In parallel, MD simulations illustrated the relevant mechanistic step of nanoparticles formation. The aggregation of drug molecules at the bubble interface was observed and considered as an early stage of NPs nucleation.

Conclusions

In the study, the sonochemical method was applied for the production of therapeutic polyurethane material. This method was proposed as an alternative, more effective for preparing hybrid systems with the function of controlled in-site drug release. The principal benefits of the proposed method are: short preparation time, increased drug availability for the targeted tissue, lack of chemical waste and toxic solvents. For the investigated bioactive molecules no changes in chemical structure, activity and bioavailability upon sonochemical synthesis were observed. The obtained results open the door for the rational development of hybrid NPS/polymer implantable materials.

Acknowledgments

This study was financed by the Polish National Science Centre project awarded by decision number DEC-2019/35/D/ST5/03107.

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POLY(SEBACIC ANHYDRIDE) MICROPARTICLES LOADED WITH CURCUMIN FOR PULMONARY PURPOSES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 43]

Introduction

Fast biodegrading polymeric microparticles (MP) used as the inhaled drug delivery systems (DDS) are considered a superior treatment method for pulmonary infections, allowing to obtain the therapeutic effect at lower antibiotic doses, reduced side effects, and smaller chances of developing antibiotic resistance [1].

The common problem of using MP as DDS is the need to use a relatively big amount of them due to limited drug loading capacity. This issue may possibly be solved by the use of quorum sensing inhibitors (QSi) e.g. curcumin (CU) – commonly applied in the food industry as a coloring factor. QSi prevent bacteria from creating biofilms, making them more sensitive to antibiotics [2].

Herein, we present the study of CU-loaded MP from poly(sebacic anhydride) (PSA) – a polymer investigated for drug delivery due to its favorable degradation kinetics. The aim was to evaluate the entrapment efficacy of CU in the MP and to assess the influence of encapsulated CU on the cytotoxicity of the system in contact with lung epithelial cells.

Materials and Methods

PSA was obtained from sebacic acid via polycondensation. MP were manufactured using oil-in-water (O/W) emulsification, where; O: PSA and CU in different ratios dissolved in dichloromethane (DCM); W: water solution of poly(vinyl alcohol) (PVA). CU at different ratios was dispersed in PSA solution using ultrasounds. MP were obtained by adding the oil phase to the water phase, and then the organic solvent was evaporated under constant stirring. MP were washed 3 times in UHQ-water to get rid of surfactant residues, and freeze-dried. MP were observed using optical microscopy.

CU entrapment efficacy was evaluated by the fluorometric study of the residual water phase diluted in dimethyl sulfoxide (DMSO) at excitation wavelength 485-412 nm and emission wavelength 590-510 nm. Cytotoxicity was determined using AlamarBlue assay on human lung epithelial cells (BEAS/2B) after 24 h contact with MP dispersed in Dulbecco modified Eagle's medium (DMEM) at different concentrations from 0.1 µg/ml to 1000 µg/ml (n=3 for each concentration; analyzed by Tukey HSD test).

Results and Discussion

Obtained MP were round in shape with diameter sizes below 5 µm and yellow coloration from encapsulated CU (FIG. 1). The experiment showed that the addition of CU up to 10% of PSA mass results in entrapment efficacy around 55% of initial values (54.60 ± 1.01% and 54,98 ± 2.58% for 5% and 10% CU, respectively) and decreased at higher concentration (42.76 ± 0.70% for 20% CU). Although the efficacy decreased, the MP loading increased with the concentration up to around 11% of MP mass. Obtained CU loadings are very promising for future combinations with antibiotics and prove the ease of manipulating the amount of entrapped CU within PSA MP.

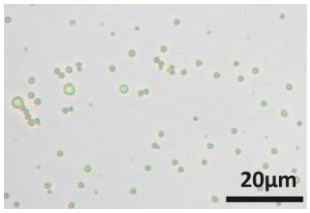

FIG. 1. 20% CU-loaded MP under optical microscope

AlamarBlue assay showed no cytotoxicity of empty or CU-loaded MP at concentrations lower or equal to 10 µg/ml. At 50 µg/ml, significant decrease in cell metabolism appear in both types of MP. In the case of CU-loaded MP the decrease in cell activity seems to be lower (differences at concentration 50 µg/ml are at $p<0.001$ for empty MP and $p<0.01$ for CU loaded MP) (FIG. 2).

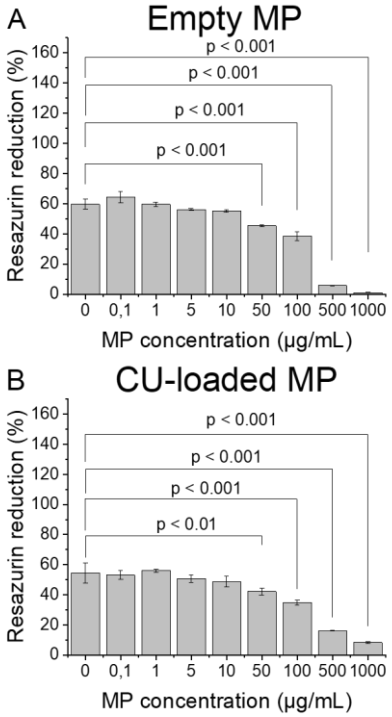

FIG. 2. BEAS/2B cell viability cultured in contact with Empty MP (A) and 20% CU-loaded MP (B); (error bar: SD, n=3).

Conclusions

This study showed a great potential of CU as a QSi to be administrated via inhalation in polyanhydride MP-based delivery systems. The final drug loading may easily be controlled by changing the initial concentration of CU. CU entrapment does not increase the cytotoxicity of PSA MP. It is even possible for CU to make the MP more less cytotoxic, but this hypothesis has to be proved by further studies.

Acknowledgments

This study was supported by the National Science Centre, Poland (project No 2019/35/B/ST5/01103) and by the Program "Excellence Initiative – Research University" for the AGH University of Science and Technology.

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POLY(L-LACTIDE-*CO*-GLYCOLIDE) MEMBRANES MODIFIED WITH RGD-POLY-(2-OXAZOLINE) FOR BIOMEDICAL APPLICATION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 44]

Introduction

The demand for biomaterials for dental applications is still growing [1]. POx as well as arginine, glycine and aspartic acid sequences (RGD) are considered as very powerful molecules, which can change properties of membranes and improve cell adhesion [2]. The aim of this study was to prepare and evaluate the properties of a surface-modified resorbable poly(L-lactide-*co*-glycolide) (PLGA) membrane for guided tissue regeneration (GTR). For this purpose, we used poly(2-oxazoline) (POx) grafted with cell adhesive RGD sequences to modify the surface of PLGA membranes. The membranes were produced by one-step phase separation process between PLGA, poly(ethylene glycol) (PEG) and POx_RGD (or POx), dissolved in dichloromethane (DCM) followed by PEG leaching.

Materials and Methods

PLGA (85:15, Mn=100 kDa, d=1.9), PEG (400 Da), POx (methyl-P[MeOx37-b-BuOx-23-b-MeOx37-pipeidine(P2-P2) (Mn=8 kDa,d=1.14) or RGD-grafted POx (POx_RGD) were dissolved in DCM, casted on Petri dishes, dried in air and under vacuum, followed by soaking in UHQ-water for PEG leaching to obtain the membranes (M_POx) and (M_POx_RGD).

PLGA membranes (M) and PLGA foils without POx (Foil) as reference materials were prepared. Microstructure of the samples was observed under scanning electron microscopy (SEM). Water contact angle, Raman spectroscopy and Fourier transform infrared spectroscopy (FTIR) were used to characterise the materials. Cytocompatibility tests were performed with osteoblast-like MG-63 cells for 4, 24 and 72 h. Viability was measured by resazurin reduction; phalloidin/DAPI and live/dead staining tests were done.

Results and Discussion

Phase separation between PLGA and PEG followed by PEG leaching was found useful in producing porous asymmetric membranes. Addition of POx and POx-RGD to the system influenced topography of the M_POx and M_POx_RGD, by changing average pore size on the upper and lower sides of the membrane (FIG. 1). Addition of POx and POx_RGD also influenced wettability of the membranes and reduced water contact angle as compared to foil and non-modified membrane (FIG. 2). Application of Raman and FTIR spectroscopy allowed for characterization of chemical differences occurring between the investigated membranes (data not shown).

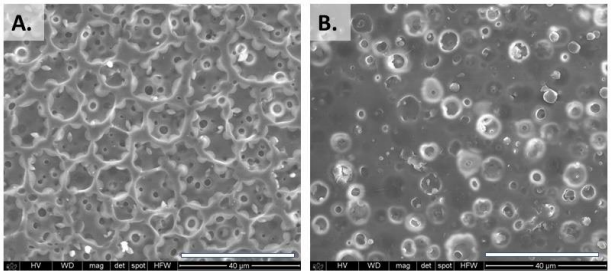

FIG. 1. SEM morphology of M_POx_RGD membrane, upper (A) and lower (B) side. Scale bar = 40 µm. Magnification 2000x.

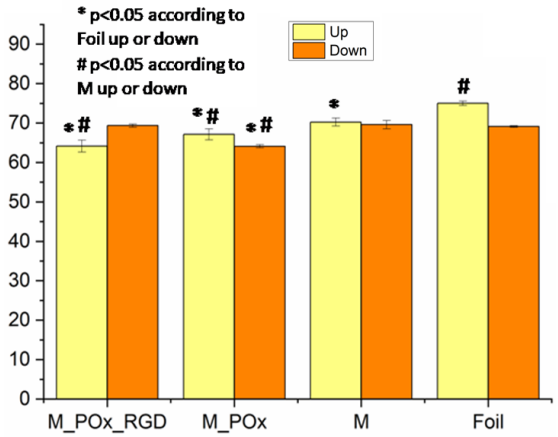

FIG. 2. Results of wettability for M_Pox_RGD membrane, M_POx membrane, PLGA membrane (M) and PLGA foil. Level of significance at the level * p<0.05 according to Foil up or down and membrane up or down.

Conclusions

One-step phase separation process resulted in M_POx_RGD membranes, which were found cytocompatible with osteoblast-like MG-63 cells. M_POx_RGD supported adhesion of the cells as compared to foil and M. Obtained membranes can be considered for GTR in stomatology, periodontology and in bone tissue engineering.

Acknowledgments

This study was realised in the frame of Interfaculty Environmental Doctoral Studies (FCB) at the Faculty of Materials Science and Ceramics, AGH-UST and the Institute of Nuclear Physics, Polish Academy of Sciences. This study was supported by the Program "Excellence Initiative – Research University" for AGH-UST and from the subsidy of the Ministry of Education and Science for AGH-UST (No. 16.16.160.557). This research was performed using equipment purchased in the frame of the project co-funded by the Małopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project No. MRPO.05.01.00-12-013/15.

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STRUCTURE-PROPERTY CORRELATION STUDIES OF CROSS-LINKED CITRIC ACID-BASED ELASTOMERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 45]

Introduction

In recent years, poly(alkylene citrate) (PAC) biomaterials have become one of the most promising biomaterials for the production of scaffolds for soft tissue engineering [1]. Moreover, they were considered as components of numerous composites and copolymers studied in a wide range of possible medical applications. In terms of mechanical properties, PAC-based materials are similar to those of human tissues and can be readily tailored to specific needs [2] thus providing the opportunity of being adopted to a variety of bioengineering applications. In addition, cell proliferation studies indicated their remarkable biocompatibility and absence of cytotoxicity, with a significant decrease in thrombogenicity [3]. However, challenging structural characterization of PAC prepolymers has been reported in only a few reports despite the extensive published literature [4], with the majority of papers showing serious incoherencies in the discussion of the reported spectroscopic results. Hence, the main focus of this work was to optimize the composition and synthesis parameters of PAC polymers based on two diols: 1,6-hexanediol or 1,8-octanediol in two comonomer molar ratios, to obtain materials with defined structure and physicochemical properties. For evaluating their suitability as biomaterials, experiments were performed with adipose tissue-derived stem cells.

Materials and Methods

PAC prepolymers were synthesized in polycondensation reaction of citric acid and 1,8-octanediol (poly(octamethylene citrate) (POC)) or 1,6-hexanediol (poly(hexamethylene citrate) (PHC)) in a molar ratio of 2:3 or 1:1, at 140^{o}C for 40 min, and later they were purified and freeze-dried. The prepolymers were characterized using 1D and 2D NMR techniques, mass spectrometry (ESI-MS) and acid value determination. Cross-linked PAC materials (cPAC) were obtained after postpolymerization of 30% ethanolic solutions of the prepolymers in specific molds (4 or 10 days at 80^{o}C). The cPACs were characterized according to their mechanical properties. Evaluation of biological properties of the materials were conducted both on extracts of the materials and in direct contact using adipose tissue-derived stem cells.

Results and Discussion

NMR and MS results indicated the priority of the formation of linear oligomers, which directly translates into cross-linking density as well as mechanical and biological properties of the materials obtained. Our research also confirms the validity of using NMR analyses to determine the fundamental properties of cross-linked materials as early as at the stage of prepolymer synthesis. The values of tensile strength and maximum tensile strength at break of cPACs increase significantly with increasing cross-linking time while the reversed trend was observed for relative elongation and elongation at break. On the other hand, cPOC exhibit lower values of tensile strength and higher elasticity than cPHC materials which can be explained by the differences in chain length in the diol used for synthesis. The obtained results show differences in properties between the materials fabricated in 1:1 and 2:3 molar ratio in spite of significantly higher tensile strength values and decreased elongation for the latter materials. The first approach towards assessing cell behaviour in indirect contact with PAC polyesters was performed with the use of adipose tissue-derived stem cells (ASCs) (calcein-AM/propidium iodide counterstaining). Cells cultured within undiluted extracts were found dead or at the beginning of cell death, excluding cPHC_2:3_10d, where they were well spread. 5% extract from cPOC_1:1_10d also resulted to be fatal for ASCs. Their morphology and distribution on day 1 were well-defined and such parameters did not vary from cells cultured in control conditions. For *in vitro* test in a direct contact with ASCs, we selected cPHC_2:3_10d and cPOC_2:3_10d. On days 1 and 3, the cell number and morphology were similar on both studied polymers and on TCPS.

Conclusions

In this work, we presented structure-properties correlations of PAC biomaterials. Detailed NMR spectra combined with MS and acidity determinations allowed for a thorough understanding of the PAC structure, explanation of chemical nature of the synthesis process and shed a light on the chemistry of the material cross-linking process. The final and the most relevant conclusion of the presented paper is the correlation of the acidity, and the molar ratio of reactants with cell viability and proliferation studies results performed on material extracts. The results indicate the privilege of using a 2:3 molar ratio of reagents than commonly described in the literature 1:1 ratio while maintaining all the properties of the latter. In vitro tests performed in a direct contact with cells show that the final biological output can also be tuned by using diols of a higher number of carbon atoms in the chain.

Acknowledgments

This study was financed by National Science Center, Poland (2018/28/C/ST5/00461), by the Program ”Excellence Initiative – Research University” and from the subsidy of Ministry of Education and Science for AGH-UST (No. 16.16.160.557). Further support was provided by the Czech Science Foundation (grant No. 21-06065S). Martina Trávníčková received funding (Development of HR capabilities, internationalization, popularization and IP utilization, No. CZ.02.2.69/0.0/0.0/16_028/0006226) for traveling to Poland.

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POLY(L-DOPA)-MODIFICATION OF CURDLAN HYDROGEL FOR SAFE AND EFFECTIVE DRUG BINDING AND RELEASE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 46]

Introduction

Observation of clams adhesion to many types of surfaces contributed to significant progress in the surface modification area. It has been shown that the main agent responsible for this extremely strong adhesion are dopamine and proteins lysine-rich. PDA (polydopamine) coating has the ability to deposit on many types of substrates and has ability to increase their functionality [1]. Covering the surface of biopolymers with a polycatecholamine layer significantly improves their functionality [2,3]. However, L-DOPA being a dopamine precursor in the biochemical synthesis pathway (with free carboxyl group in its structure) also forms highly adhesive coatings but it is very rarely used for surface functionalization [4]. Therefore, we decided to make a wide-ranging characterization of matrices obtained using L-DOPA as a curdlan modifier.

Materials and Methods

Functionalization of curdlan hydrogel was performed by L-DOPA polymerization from 2 or 4 mg/ml solution for 24 h at 25ºC. L-DOPA monomer was added to curdlan matrix before its gelation (sample 2-LD-BG and 4-LD-BG) or after (2-LD-AG). The matrices were characterized using the FTIR and XPS methods and the soaking capacity was evaluated in defined time points. The biological safety of the modified hydrogels was verified after contact with blood, fibroblasts cell lines and in zebrafish model, in relation to their possible application as wound dressings. Secondly, modified hydrogels were coupled with gentamicin and the amount of bound drug was estimated spectrophotometrically, based on gentamicin derivatization by phthaldialdehyde. Drug release from each variant of modified hydrogels was evaluated by incubation in PBS pH 7.4 at 37ºC, with daily exchange of buffer and replacement it by the new portion. Antibacterial activity of functionalized curdlan matrices was evaluated by indirect method, in extracts collected in similar way as in drug release test (PBS was replaced by Mueller-Hinton Broth). The extracts were inoculated by three reference bacterial strains and allowed at 37ºC for 24 h. Then the bacterial growth was estimated in daily collected extracts as optical density at 660 nm in Synergy H4 plate reader. The samples of hydrogel after incubated with MH Broth medium were used to indirect bacterial adhesion test. Experiments were performed in triplicate.

Results and Discussion

Blackish colour of modified hydrogels was the indicator of the successful deposition of the functional poly(L-DOPA) coating. XPS also confirmed the presence of poly(L-DOPA) coating on the hydrogels. The increased soaking capacity of poly(L-DOPA)-modified curdlan hydrogels manufactured by AG method seemed to be related to the specific method of curdlan modification (where L-DOPA monomer was introduced into curdlan matrix after curdlan gelling process). Poly(L-DOPA)-modified curdlan hydrogels were stable in blood and plasma as well as in the wide range pH buffers (excluding alkaline media). Poly-levodopa coatings on curdlan were nontoxic in zebrafish model and did not negatively change the crucial blood parameters. Modified hydrogels were nontoxic for primary fibroblasts cultures and inhibited their adhesion for some modification variants. All modified hydrogels showed ability to bind gentamicin and exhibited high antibacterial properties.

Conclusions

We assumed that coating formed by L-DOPA may impart new beneficial features of the curdlan hydrogels and that kind of modification is safe and promising for future curdlan medical applications as wound dressings. Use of L-DOPA monomer for curdlan modification process allows for a more efficient drug binding in comparison with dopamine for that purpose (Michalicha et al., 2021).

Acknowledgments

This study was financed from DS6/2021 grant (Medical University of Lublin, Poland).

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EFFECT OF TEXTURED SURFACE TOPOGRAPHY PARAMETERS ON CELLS PROLIFERATION AND TISSUE GROWTH IN THE MECHANICAL CIRCULATORY SUPPORT APPLICATION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 47]

Introduction

Recently, the number of patients with heart failure has been increasing. The first step is to perform pharmacological treatment. However, when such treatment does not bring the expected effects, there may be a need to implement mechanical circulatory support (MCS). One possible solution is to use blood pumps such as Ventricular Assist Devices (VAD) [1]. These devices are nowadays widely used as an effective treatment for patients with advanced heart failure and are the only alternative to heart transplantation. Currently, the most commonly used VAD constructions in clinics are fully implantable. Therefore, it is very important to provide their high biocompatibility to ensure their long-term use, patient safety and comfort. Over the years many new devices were introduced to the clinic [2]. However, based on clinical experience, despite undeniable evidences of this treatment effectiveness, many components could still be improved. Most of the currently used blood pumps are made of titanium alloys. The ongoing optimization performed by many producents does not concern only improvements in terms of their function, but also in terms of patient comfort and reduced mortality. According to the information provided by INTERMACS, patient survival drops to 50% after four years of support. However, the VADs, are designed to be wear-resistant and in some cases are used as destination therapy. It is therefore important to further improve the devices biocompatibility in order to reduce the occurrence of complications [3,4]. One of possible complications may be the pump thrombosis and inflow obstruction, caused by the ingrowth of tissue into the lumen of inflow cannula [5-7]. According to reports from clinics and literature, it seems that the solution to this problem may be the use of surface modification [8]. Appropriate topography allows controlled scar tissue formation, which results in reduction of inflammatory processes and the appearance of thromboembolic events [9-10].

Materials and Methods

The paper presents surface modification performed using vacuum sintering intended for use in an VAD inflow cannula. The study presents an analysis of the relationship between surface parameters on the susceptibility of cells to proliferate and the strength of their adhesion to the implant. Samples were prepared from titanium alloy Ti6Al7Nb in form of cylinders Ø14 mm x H 3 mm. During the initial research, the base material was verified for compliance with the standard including the microstructure study, the chemical composition analysis and the study of mechanical properties. Then samples were subjected to tumbling before performing modification. The initial roughness was measured with the use of contact profilometry and was characterised by Ra = 1,5 µm and Rz = 12.5 µm. The sintering process included modifications with the use of Comercially Pure Titanium (Cp-Ti) powder with two different grain morphologies - spherical and irregular. The grain size was changed in rage of 50 to 250 µm. The obtained surfaces were then analysed by scanning electron microscope [SEM]. Additionally, the porosity of obtained surfaces was determined. All samples revealed high roughness with the potential for cell anchoring and scar tissue formation. Fibroblasts were then applied to the samples for three periods of time. The number of cells was assessed on the basis of the stained cell nuclei and the presence of adhesive molecules. In addition, in vivo studies were performed in which samples were implanted into the dorsal muscle of New Zealand rabbits. After 4 and 8 weeks, the specimens were deplanted and the force of detachment of formed tissue from the implant surface was tested. Then the tissue sections were analyzed for inflammatory reactions and histopathology.

Results and Discussion

The results have shown that surface after powder sintering is characterized by high porosity and complex 3D morphology. The obtained roughness was in rage Ra = 21-36 µm. The porosity was in rage 27-49% depending on the size and shape of the powder grains. The permanent connection was obtained at the implant-muscle tissue interface as a result of the surface modification. The detachment force differed by 0.5N depending on the shape of used powder grains.

Conclusions

The presented modification has the potential to anchor cells and form controlled scar tissue on the surface of the implant, both in the context of cardiac and other medical implants.

Acknowledgments

NCBiR: RH-ROT/266798/STRATEGMED-II
National Science Centre, Poland: 2018/31/N/ST8/01085

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EMULSION ELECTROSPINNING - METHOD TO INTRODUCE PROTEINS FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 48]

Introduction

The possibility of manufacturing of nanofibrous scaffolds mimicking the microstructure of the extracellular matrix (ECM) is an advantage of electrospinning techniques used in tissue engineering and regenerative medicine. Modification of the electrospinning conditions as well as the possibility to modify the solution used to produce nanofibers give new opportunities to prepare scaffolds with desired behaviour in the organism. One of the modifications used to release biologically active compounds or drugs is emulsion electrospinning. The advantage of this solution is the control over the release kinetics of the active ingredient or biomolecule and the high-efficiency encapsulation [1]. Moreover, encapsulation of proteins and pharmaceuticals in electrospun fibers is one of the strategies to overcome the barriers associated with maintaining the stability and effectiveness of the active ingredient during the formulation process [2].

In our study, we focused on the possibility of loading protein into nanofibers using emulsion electrospinning, which creates core-shell nanofibers from an emulsion consisting of an organic phase (polymer) with surfactant and aqueous phase. To prevent negative interaction of the model protein (albumin, alanine) suspended in the aqueous phase with the polymer organic solvent (PCL) during the electrospinning process, commercial surfactants used in cellular research (Tween 80, Triton X100) were used. The next step was to develop electrospinning conditions to obtain fibers enriched in encapsulated proteins. Specifically, we investigated the solution and emulsion process parameters for electrospinning to achieve high protein loading into nanofibers, preservation of protein bioactivity prolonged formulation stability, and controlled protein release for future biomedical applications.

Materials and Methods

Both oil and water phases for emulsion were prepared separately. 15% (w/v) Polycaprolactone (PCL, Mw = 80kDa, Sigma-Aldrich, Germany) and surfactant (Tween80, Sigma-Aldrich) at different concentrations (0,1%, 0,5% , 1% v/v) were dissolved in dichloromethane (DCM, Chemland SA) and dimethylformamide (DMF, Chemland SA). Alanine (L-alanina, Sigma-Aldrich, Germany), as well as Bovine Serum Albumin (Mw = 64kDa, Sigma-Aldrich, Germany), were dissolved in a distilled water to prepare a water phase. Then, both alanine and albumin solutions were dropwise added to the polymer solution and stirred in an ice bath. The microstructure of nanofibers was observed with a scanning electron microscope (NOVA NANO SEM 200). To determine the physicochemical properties of a scaffold, contact wet angle and surface free energy were measured with a goniometer (DSA 25 Kruss). The presence of the surfactants was checked by ATR mode in FTIR study (BioRad Tensil 60). The release kinetics of peptides in the material was assessed by turbidimetry (2100AN IS Laboratory Turbidimeter). Cytocompatibility was assessed by seeding fibroblasts on a fibrous scaffold after 7 days of contact with the fibrous scaffold.

Results and Discussion

The results of the study confirm that the surfactant addition strongly affects the physicochemical properties of the fiber surface; the hydrophobicity decreases and the fibers from superhydrophobic materials become hydrophilic. As shown in FIG. 1 the contact angle of pure fibrous PCL decreases from 130° to about ≈20° for PCL fibers obtained from PCL/Triton, PCL/Tween mixture. The observed effect is independent of the surfactant concentration. This is proved by the hydroxyl bands in the range 3500 cm^{-1} and 1190 cm^{-1} typical for the structure of both surfactants. On the other hand, a change in the ratio of the 1190:1170 cm^{-1} bands is observed in materials where triton/albumin and triton/alanine emulsions were introduced into the fibers during the electrospinning process. Protein macromolecules encapsulated in micelles do not yield bands characteristic of the proteins used, it is cover by surfactants layer. Surfactant addition does not affect the diameter of PCL fibers, whose distribution ranged from 280-1800nm for pure PCL and remained unimodal with a maximum at 1000-1500 nm for PCL/Tween80. In contrast, the addition of emulsion to the spinning solution with PCL resulted in a significant increase in the fiber size, whose distribution remained unimodal but the range widened to 3000 nm (for PCL/Tween80/albumin). The observed changes are a consequence of the larger micelle sizes present in the emulsion. None of the tested scaffolds showed a cytotoxic effect.

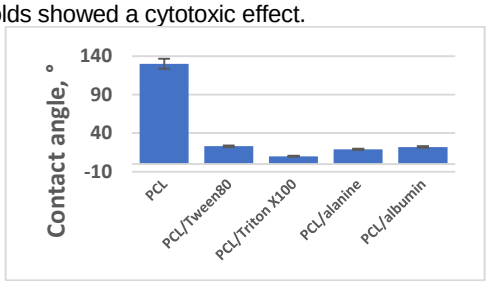

FIG. 1. Contact angle of the samples.

Conclusions

The emulsion electrospinning process allows obtaining fibres modified by micelles with biological compounds. The addition of the surfactants as well as surfactants with encapsulated protein increase hydrophilicity and diameter of polymer fibres. Moreover, the presence of an external layer of a polymer in a shell part provides more controllable kinetics of biomolecules release. Neither surfactants nor micelles with surfactants and proteins did not induct cytotoxicity of fibrous scaffolds.

Acknowledgments

This work was supported from the subsidy of the Ministry of Education and Science for the AGH University of Science and Technology in Kraków (Project No 16.16.160.557).

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BIOACTIVE HYDROCOLLOID-TYPE BIOMATERIALS FOR POTENTIAL MANAGEMENT OF HIGHLY EXUDING WOUNDS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 49]

Introduction

The problem of treating chronic wounds that are difficult to heal is common in regenerative medicine. Thus, bioactive wound dressings are applied more often to accelerate the healing process. Recently many trends in the production of dressing materials composed of natural polymers are observed in the engineering of biomaterials field [1]. It has become very common to use β-glucans (natural immunomodulators) as a matrix for the production of biomaterials for wound care. Special attention has been paid to curdlan (β-1,3-glucan), which has anti-inflammatory properties and supports wound healing [2]. The main purpose of this work was to create superabsorbent biomaterials with typical hydrocolloids properties for the care of exudative wounds. Within this study, curdlan has been combined with other natural polymers such as agarose or chitosan to create hybrid biomaterials for exuding wound management [3].

Materials and Methods

The curdlan/agarose biomaterial (marked as Cur/Aga) was prepared by mixing the appropriate ratio of curdlan and agarose. The polymers were dissolved in deionized water at high temperature, creating a gel. The curdlan/chitosan material (marked as Cur/Chit) was composed of the suitable proportion of curdlan and chitosan suspended in acetic acid. After obtaining homogeneous mass, the mixture was transferred to containers and incubated in a water bath at 95°C for 20 min. Afterwards, the Cur/Chit material was neutralized in NaOH. Both samples (Cur/Aga and Cur/Chit) were frozen at -80°C for 1-2 hours and subjected to lyophilization process for 16 hours. The chemical composition of the biomaterials and their production methods were described in details in Polish Patent no. 236367, 2021 (Cur/Aga) and patent application no. P.430455, 2019 (Cur/Chit). The resulting foam-like materials were subjected to further tests.

The cell culture experiments were carried out using human normal skin fibroblasts (BJ) obtained from ATCC. To evaluate cytotoxicity of the produced foam-like biomaterials, an indirect test was conducted in accordance with ISO 10993-5 (2009). Cell viability next to and on the biomaterials was evaluated by Live/Dead staining. Moreover, biocompatibility tests (cell proliferation and collagen synthesis) were performed using a two-compartment model with cell culture inserts. Tested samples were placed into the wells of multiwell plate, however BJ cells at a concentration of 1×10^4 cells were seeded into the inserts. The cells were cultured for few days and then cell number was specified using WST-8 assay, whereas collagen synthesis was visualized by immunofluorescence using human-specific anti-type I collagen (Col1a1/Col1a2) antibodies with secondary antibodies conjugated to Alexa Fluor 647. Images were acquisitioned using CLSM.

Assessment of exudate absorption ability of biomaterials was carried out by immersion of the samples in human blood plasma and serum. At specified time intervals, the samples were removed from the physiological fluids, weighted, and put back in the liquid.

Results and Discussion

MTT cytotoxicity assay showed that the developed materials were not-toxic to human skin fibroblasts. Compared to the control, cell viability was not under 83 % after 48 h incubation of the cells in the presence of Cur/Aga and Cur/Chit extracts. Visualization of fibroblasts by CLSM showed monolayer of viable BJ cells around the materials and only single spherical cells (unattached) on the surface of tested biomaterials, which means, that their surface prevents adhesion of skin fibroblasts. Based on the results of biocompatibility tests, it may be concluded that tested biomaterials did not have a negative effect on cell proliferation and type I collagen synthesis. Foam-like biomaterials were highly absorbent and their structure changed to the gel after contact with the exudate, acting as the hydrocolloid material (FIG. 1).

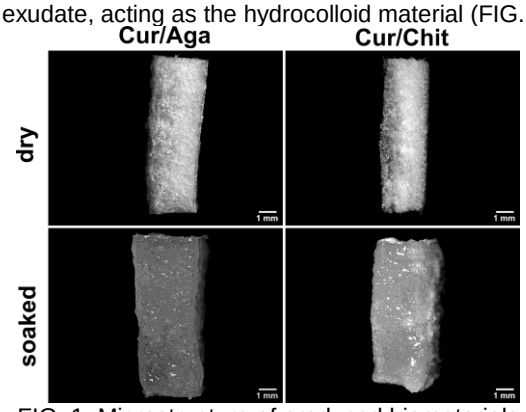

FIG. 1. Microstructure of produced biomaterials (scale bar = 1 mm).

TABLE 1. The exudate absorption capacity of tested biomaterials presented as volume [µl] of plasma absorbed by 1 g of the biomaterial.

Sample	Time [s]			
	3s	6s	12s	21s
Aga/Cur	8382.3 ± 979.3	10162.5 ± 728.6	11447.4 ± 282.7	11905.9 ± 701.2
Aga/Chit	3546.6 ± 1304.6 *****	7551.3 ± 779.4 *****	10799 ± 1448.9	11752 ± 1225.4

*statistically significant results compared to Aga/Cur; $P < 0.05$, unpaired t-test

Conclusions

Obtained results demonstrate that fabricated biomaterials are characterized by high biocompatibility. However, they hinder adhesion of human skin fibroblasts to their surface, allowing for painless removal of the dressing after healing. The foam-like biomaterials have the ability to transform into typical hydrocolloid dressings with superabsorbent properties after contact with physiological fluids. All mentioned features of the biomaterials prove their promising potential to be applied for highly exuding wound management.

Acknowledgments

The study was supported by National Science Centre (NCN) in Poland within OPUS 16 grant no. UMO-2018/31/B/ST8/00945.

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SUPERABSORBENT VITAMIN C-ENRICHED CHITOSAN-AGAROSE BIOMATERIAL FOR EXUDING WOUND MANAGEMENT

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[ENGINEERING OF BIOMATERIALS 163 (2021) 50]

Introduction

The new approach to wound repair combines modern strategies consisting of the application of progressive wound dressings that are incorporated with bioactive molecules, promoting wound contraction and re-epithelialization [1]. The aim of the study was to develop new production method of highly absorbent chitosan-agarose biomaterial enriched with vitamin C that may be potentially used as a bioactive dressing for the management of exuding acute and chronic wounds.

Materials and Methods

Chitosan/agarose biomaterial was prepared by mixing agarose solution obtained by heating agarose powder in sodium hydroxide solution at 95°C with chitosan solution obtained by dissolving chitosan powder in acetic acid. Both solutions were mixed until homogeneous mass was obtained. In the next step, the chitosan-agarose mixture was cooled, appropriate vitamin C amount was added, and the entire content of the beaker was mixed. Obtained mass was spread on the polystyrene surface, frozen and lyophilized. Importantly, production method of the porous chitosan-agarose wound dressing has been claimed in the Polish Patent Office (patent application no. P.430457).

Cell culture tests. ISO 10993-5:2009 and ISO 10993-12:2012 standards were used to evaluate the cytotoxicity of the produced biomaterial. The viability of human normal skin fibroblasts (BJ) cells obtained from ATCC was evaluated upon exposure to the biomaterial extract using MTT test. Cell viability was also determined using Live/Dead Double Fluorescent Staining Kit.

Exudate Absorption Capacity. In the estimation of the absorption capacity of the tested biomaterials, human blood plasma and serum were used. Prepared samples were immersed in the physiological fluids and after different time intervals of soaking were weighed.

Mechanical properties. Biomaterial mechanical properties were determined using an autograph AG-X plus universal testing machine. The crosshead rate in the conducted test was 50 mm per min, and ultimate tensile strength, the ultimate elongation at break, and Young's modulus were determined.

Results and Discussion

Carried out cytotoxicity test showed that all evaluated materials were non-toxic because cell viability exceeded 70% after both time intervals (24 and 48 h) (FIG. 1). Higher cell viability was noted for biomaterials with incorporated vitamin C. The lack of cytotoxicity was also confirmed in the direct LIVE-DEAD staining test, where a few spherical, non-flattened but viable cell (stained on green) were observed (FIG. 2.). It demonstrated that the tested biomaterial hindered cell growth and attachment on its surface. It was also revealed that the tested biomaterial possessed high ability to uptake physiological fluids because 1g of chitosan-agarose material absorbed 14 ml of plasma and 15 ml of serum (FIG. 3). The conducted tensile test revealed its elastic-plastic behaviour. Low Young's modulus value (0.15 ± 0.06 MPa) and very high elongation at break results (75 ± 7.5%) indicate its high elastic deformations, and good stability during its stretching, respectively.

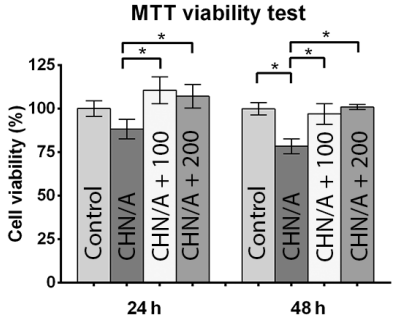

FIG. 1. MTT cytotoxicity test carried out according to ISO 10993-5 [2].

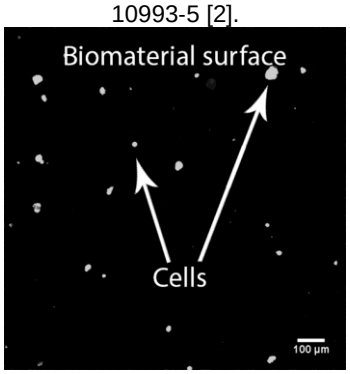

FIG. 2. Fibroblast cells growth on the biomaterial assessed by LIVE/DEAD staining [2].

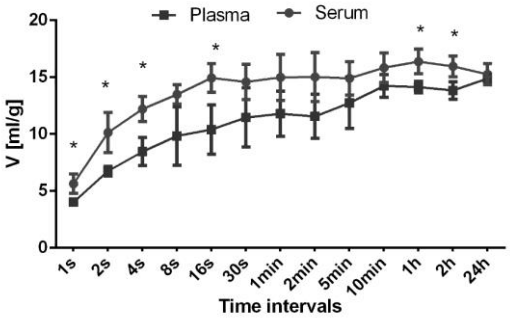

FIG. 3. Biomaterial exudate absorption capacity [2].

Conclusions

On the basis of the presented results, it can be concluded that the produced material was non-toxic and the addition of vitamin C had a beneficial effect on the cell viability. In summary, chitosan-agarose material is a great candidate that can be used as an external temporary dressing for exuding wounds that will ensure painless removal due to the lack of adherent cells on its surface.

Acknowledgments

The study was supported by National Science Centre (NCN) in Poland within OPUS 16 grant no. UMO-2018/31/B/ST8/00945 and by using the equipment purchased within agreement no. POPW.01.03.00-06-010/09-00 Operational Program Development of Eastern Poland 2007–2013, Priority Axis I, Modern Economy, Operations 1.3. Innovations Promotion.

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ADHESION, MICROSTRUCTURE AND SURFACE TOPOGRAPHY OF MESOPOROUS, Cu-DOPED SOL-GEL GLASS/ZEIN COATINGS ELECTROPHORETICALLY DEPOSITED ON Ti-13Nb-13Zr ALLOY SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 51]

Introduction

Among metallic biomaterials, titanium and its alloys are widely used in biomedical engineering. They are characterized by good biocompatibility, high electrochemical corrosion resistance, favourable fatigue strength and a high strength to weight ratio. However, they are biologically inert to the human body and biofilms are formed on their surface [1]. In order to improve the biological properties, the surface can be modified by the deposition of coatings. For this purpose, polymer-ceramic composite coatings are commonly used. In this work, composite coatings with a zein matrix, containing bioactive, mesoporous sol-gel glass (MSGG) with the addition of CuO (70% SiO_2, 25-x% CaO, 5% P_2O_5 + x CuO (x=1-3), in mol %), were produced on near-β Ti-13Nb-13Zr alloy substrates by electrophoretic deposition (EPD). EPD is an electrochemical surface modification method that allows the co-deposition of polymers and ceramics [2]. Zein is a natural polymer. Due to its biocompatibility, biodegradability and low toxicity, it can be used as a matrix material [3]. MSGG is characterized by high open porosity and a large specific surface area, which are desirable, among others, in the regeneration of bone tissue [4]. CuO has good antimicrobial properties and is widely used in biomedical engineering [5]. The aim of this work was to deposit MSGG/zein composite coatings, to investigate their adhesion to the Ti-13Nb-13Zr alloy substrate and to characterize their microstructure and surface topography.

Materials and Methods

A Ti-13Nb-13Zr titanium alloy was used as the substrate material for coating deposition. The samples were ground with 1200 grit sandpaper and then washed with ethanol and distilled water. To prepare the suspensions for the EPD process, zein powder (200 g/l) was gently added to the solutions of anhydrous ethanol (90 vol. %) and distilled water (10 vol. %), forming a zein solution to which MSGG (10 g/l or 40 g/l or 80 g/l) was gently added. The EPD was held on a two-electrode setup, where the working electrode was a Ti-13Nb-13Zr alloy and the counter electrode was an austenitic stainless steel plate (AISI 316L). The distance between electrodes was 10 mm. The deposition time was 5 minutes. The applied voltage was in the range of 3-10 V. The microstructure of the coatings was investigated by stereoscopic microscopy, scanning (SEM) and transmission (TEM) electron microscopy. The phase composition of coatings was investigated by grazing incidence X-ray diffractometry (GIXRD). The surface roughness of the coatings and the substrate was examined by optical profilometry. The adhesion of coatings to the substrate was investigated using the cross-cut tape-test in accordance with ASTM D3359-D.

Results and Discussion

The quality of coatings deposited on the Ti-13Nb-13Zr alloy substrates from suspensions containing 90 vol. % of ethanol, 10 vol. % distilled water, 20 wt.% of glycerol, 200 g/l of zein and 10 g/l or 40 g/l or 80 g/l of MSGG were varied with the applied voltage and the concentration of MSGG. Together with the increasing concentration of MSGG, the coatings became more porous. Coatings deposited with a voltage of 5 V and a deposition time of 5 minutes from all the prepared suspensions were selected for further research. It was found during the tape-tests that composite coatings had greater adhesion to the substrate than zein coatings. The adhesion class of the zein coatings was 0B (more than 65% area of the coating removed), and the composite coatings were in the range of 4B-5B (less than 5% or 0% area of the coating removed, respectively). Thus, this coating was selected for further microstructure investigation. The selected coatings deposited were dense, contained glass particles and their agglomerates had a diameter in the range of 1-10 μm and numerous pores with a diameter in the range of 5-15 μm. The coating deposited from the suspension with a concentration of 40 g/l of MSGG was selected for the microstructure investigation by TEM. It was dense, with numerous glass particles and their agglomerates homogeneously embedded in the zein matrix. The selected area electron diffraction pattern obtained from the coating exhibited an amorphous nature. The presence of closed pores with a diameter ranging from 0.1–0.3 μm in the central part of the coatings was observed. The thickness of the coating was ~10 μm. Between the coating and the substrate, a passive oxide layer with a thickness of about 50 nm was present. The GIXRD pattern revealed the presence of an amorphous zein and amorphous MSGG phase in the coating. The surface roughness of coatings had a greater roughness compared to the roughness of alloy substrates. The roughness of coatings increased with the increasing MSGG concentration in the suspension used for EPD.

Conclusions

It was shown that EPD allows composite MSGG/zein coatings to be deposited. These coatings are characterized by good adhesion to titanium alloy substrates and well-developed surfaces. The microstructure of the coatings contains separate MSGG particles or their agglomerates in the zein matrix. Further characterization of the coatings is in progress.

Acknowledgements

This work was supported by the National Science Centre, Poland (decision DEC-2018/31/G/ST5/00429).

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BIOCATALYTIC SYNTHESIS OF BLOCK COPOLYESTER AS A POTENTIAL DRUG DELIVERY SYSTEM

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[ENGINEERING OF BIOMATERIALS 163 (2021) 52]

Introduction

Pharmaceutical polymeric nanoparticles (PNP's) are of great interest of nanomedicine due to their broad potential applications including diagnostic devices, gene delivery, vaccine, and drug delivery systems [1]. Depending on the type of potential usage, final physiochemical properties of PNP's can be adjusted to meet the certain requirements especially nanoparticles size which in this field is essential. Considering various methods used in nanoparticles fabrication, precipitation seem to be to most advantageous as it allows an easy control of nanoparticles size by changing organic solvents and polymer concentration. Moreover, fast processing time and low energy consumption is also beneficial [2]. Among a large number of polymeric materials, aliphatic block copolyesters have been commonly used in the manufacturing of drug delivery systems mainly because of their biodegradability that enables controlled drug release, reduced side-effects and improved therapeutic efficiency. However, existing biodegradable polymers that are suitable for biomedical applications are restricted by the crucial requirement of biocompatibility. Driven by those facts, we decided to synthesize a new biodegradable and biocompatible copolyester composed of diethyl adipate (DA), 1,4-butanediol and dilinoleic diol (DLA) as building block monomers to produce polymeric nanocarriers using nanoprecipitation method.

Materials and Methods

The copolyester poly(butylene adipate)-co-(dilinoleic adipate) (PBA-DLA) with 70:30 wt% hard to soft segment ratio was synthesized via two-stage polycondensation method in diphenyl ether using *Candida Antarctica* lipase B as biocatalyst. Briefly, the first step was carried out under inert gas flow at atmospheric pressure and at an initial temperature of 80°C. After 1 hour, when the reaction mixture was homogeneous, the temperature was increased to 95°C and by-product was collected. Further oligomerization was conducted under pressure of 600 Torr for 21 h and after that time the pressure has been gently reduced to 2 Torr and the reaction was performed for the next 72h. Upon completion, the product mixture was dissolved in chloroform, filtered, precipitated into cold methanol, and dried in vacuo at 40°C for 24 h. Different solvents of different Hildebrandt solubility parameters (δ) have been used for nanoprecipitation method for PNP's preparation. Polymer solutions with 0.5 w/v% concentration prepared in different solvents and their mixtures (acetone, acetone/acetonitrile, acetone/dimethyl sulfoxide (DMSO), dimethylformamide (DMF), acetone/DMF). The PNP's were produced by pouring 2.0 ml of the polymer solutions into 5.0 ml of pure water. The size of PNP's was further assessed via dynamic light scattering (DLS) measurements. Cytotoxicity of PBA-DLA 70-30 copolyesters was evaluated by indirect contact method according to ISO10993-5 using mouse fibroblasts cell line L929.

Results and Discussion

The expected chemical structure of the obtained copolyester was confirmed using ^{1}H NMR analysis (FIG. 1).

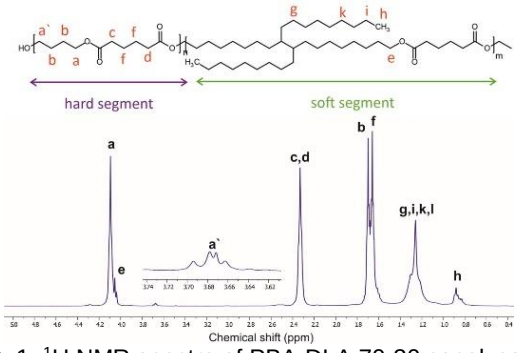

FIG. 1. ^{1}H NMR spectra of PBA-DLA 70-30 copolyester.

According to cytotoxicity studies, PBA-DLA 70-30 copolyester showed a mouse cells viability at 95% (FIG. 2).

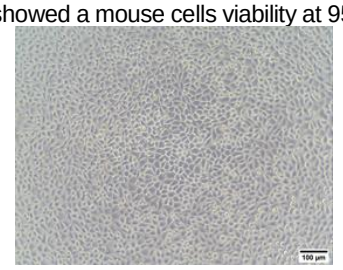

FIG. 2. Confluent mouse fibroblast cells with the presence of extracts from PBA-DLA 70-30 (magnification: 10x)

The nanoprecipitation procedure was performed by using the water miscible solvents of different δ parameter which enabled to obtain PNP's with variable size and low dispersity index similar to standards (<0.1) (FIG. 3).

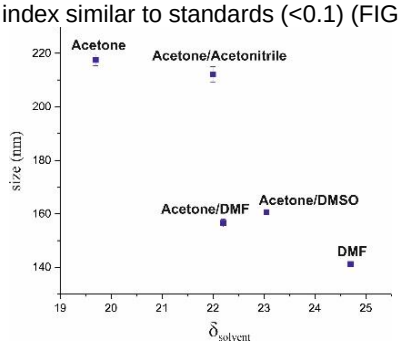

FIG. 3. Influence of the solubility parameter (δ) on the PNP's size.

Conclusions

Biobased PBA-DLA 70-30 copolyester was successfully synthesized via enzymatic polycondensation. Cytotoxicity tests revealed that material is biocompatible and exhibits minimal cytotoxicity to mouse fibroblasts. PNP's with hydrodynamic diameter ranging from 141 to 217 nm were obtained with low dispersity index (<0.1) and through nanoprecipitation. PNP's size was controlled by using different organic solvents and their mixtures.

Acknowledgments

MS acknowledges the financial support from the Dean of Faculty of Chemical Technology and Engineering.

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ELECTROPHORETIC DEPOSITION AND CHARACTERIZATION OF CuO/GtO/HA/SA COATINGS ON TITANIUM ALLOY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 53]

Introduction

Titanium and its alloys are widely used as implant material due to their interesting properties, such as good electrochemical corrosion resistance, low elasticity modulus, low density and good biocompatibility. They are the most important metallic materials used in biomedical appliances, such as bone implants in orthopedic and dental applications. However, their osseointegration properties are poor [1]. To improve biological properties, bioactive and antibacterial coatings are frequently required. In this study, composite CuO/GtO/HA/SA coatings were fabricated on near-β Ti-13Nb-13Zr alloy by electrophoretic deposition (EPD), which is a useful technique for the co-deposition of inorganic and organic components [2]. Sodium alginate (SA) is one of the most important biopolymers widely used as a matrix of composite coatings [3]. Hydroxyapatite (HA) is a well-known bioactive, osteoconductive and non-toxic ceramic material. Graphite oxide (GtO) consisting of many graphene oxide layers is an antibacterial and biocompatible material [4]. CuO has excellent antibacterial ability against various bacterial strains and is widely used in dental or orthopedic applications [5]. The aim of the present work was to elaborate the EPD conditions for the deposition of CuO/GtO/HA/SA coatings on Ti-13Nb-13Zr alloy substrates and to characterize the coating microstructure and selected properties.

Materials and Methods

A Ti-13Nb-13Zr titanium alloy was used as a substrate material for coating deposition. The substrate was ground with a successively finer grit of sandpaper up to 3000-grit and mechanically polished. The suspension used for EPD consisted of 4 g/l SA, 2 g/l HA, 0.04 g/l GtO and 0.1 g/l, 0.2 g/l or 0.4 g/l of CuO and the dispersion medium contained a volume ratio of distilled water to ethanol equal 60/40. The alloy was the anode and the cathode was made of austenitic stainless steel (AISI 316L). EPD was performed at the constant voltage of 3, 5, 7 and 10 V for a deposition time of 5 minutes. The zeta potential was measured with Laser Doppler Velocimetry in the pH range of 3 to 12. The microstructure of coatings was characterized by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The adhesion of the coatings to the substrates was investigated by a cross cut adhesion test in accordance with ASTM D3359-B. The surface topography of the coatings was analyzed by optical profilometry. The contact angle and surface free energy (SFE) were investigated with a goniometer. The antibacterial properties of the coated alloy were evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli* by using Alamar blue assay.

Results and Discussion

The zeta potential of the real suspensions, containing different concentrations of CuO equal 0.1 g/l, 0.2 g/l and 0.4 g/l, exhibited negative values in the entire investigated pH range (3–12). The highest values of zeta potential of suspensions with different concentrations of CuO were -24.5 mV (for pH=7.45, CuO 0.1 g/l), -31.0 mV (for pH=7.70, CuO 0.2 g/l) and -34.1 mV (for pH=7.90, CuO 0.4 g/l), respectively. Macroscopically uniform coatings were obtained from all investigated suspensions at the voltage of 7 V and deposition time of 5 min. If the voltage was higher than 7 V the substrate was oxidizing, but if the voltage was lower than 7 V the coatings were not uniform and only partially coated the alloy. SEM investigations revealed that coatings were smooth, homogeneous and dense with the presence of HA and CuO agglomerates. It was observed that with the increasing concentration of CuO in the suspension (0.1 g/l, 0.2 g/l and 0.4 g/l) the diameter of agglomerates was higher, 7 μm, 10 μm and 13 μm, respectively. The coatings exhibited good adhesion to the alloy substrates (class 4B, according to ASTM B3359B) independent of the concentration of CuO in the suspension. The coatings were characterized by average surface development, for example Ra (the average roughness) = 0.14 ± 0.03 μm, Rq (the root mean square roughness) = 0.21 ± 0.04 μm and Rmax (maximum vertical distance between the highest and lowest point) = 8.8 ± 2.2 μm for the coating deposited from the suspension containing 0.2 g/l of CuO. The coatings exhibited a hydrophilic character. For instance, the contact angle of coatings deposited from the suspension containing 0.2 g/l of CuO with water and diiodomethane equaled 12.2 ± 0.4° and 37.6 ± 1.5°, respectively. The SFE equaled 75.6 ± 1.1 mN/m (40.8 ± 0.7 mN/m for the disperse component and 34.8 ± 0.4 mN/m for the polar component). The CuO/GtO/HA/SA coatings deposited from a suspension containing 0.4 g/l CuO showed enhanced antibacterial activity against Gram-negative *E. coli*, comparing with the HA/SA coating obtained from a suspension without GtO and CuO. In case of Gram-positive *S. aureus* no significant difference between the coatings deposited from the suspension containing 0.4 g/l CuO and those deposited from the suspension without GtO and CuO can be found.

Conclusions

Composite CuO/GtO/HA/SA coatings were successfully deposited on titanium alloy. Macroscopically uniform coatings were obtained at a potential difference of 7 V during 5 min. All CuO/GtO/HA/SA coatings exhibited high adhesion to the alloy substrates. Coatings were dense, exhibited average surface development and a hydrophilic character. Further optimization and characterization of the coatings are in progress.

Acknowledgments

This work was supported by the National Science Centre, Poland (decision DEC-2018/31/G/ST5/00429). The authors also acknowledge support from the German Science Foundation (DFG) (project BO 1191/25-1).

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PHYSICOCHEMICAL PROPERTIES OF THE SURFACE OF LIGHT-CURED DENTAL COMPOSITES AFTER THEIR MODIFICATION WITH LIQUID RUBBER

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[ENGINEERING OF BIOMATERIALS 163 (2021) 54]

Introduction

There are many ways to improve the mechanical properties of light cured dental composites, including the use of spherical-shaped reinforcement particles [1], whiskers [2], or glass fibers [3]. Increasing the fracture toughness of composites is also possible due to the modification of matrix resins by introducing liquid rubber [4-6]. The presence of liquid rubber in the matrix of these composites, in addition to reducing shrinkage and improving mechanical properties, can also positively affect the hydrophobic and biological properties by changing the surface condition [7]. This work aims to assess the contact angle and surface free energy of two types of commercial composites (flow and condensable) before and after modification of their matrix with liquid rubber.

Materials and Methods

Two commercial composites: Flow-Art and Boston (Arkona) was used for modification and testing. The matrix was a mixture of dimethacrylate resins: Bis-GMA, TEGDMA, UDMA and EBADMA. Composition of the mixture was completed by addition of photoinitiator, stabilizer and inhibitor. Both types of composites contained the same ceramic filler which was a mixture of Ba-Al-B-Si glass, pyrogenic silica and titanium dioxide. The flow type composite contained 60% ceramics by weight of polymer matrix, while packable composites contained 78% wt. of reinforcement. The exact amounts of ingredients and their composition were patented by the manufacturer (Arkona). The modification of RBC's was made by the addition of 5% by weight (of resin) of a liquid methacrylate-terminated polybutadiene Hypro® 2000X168LC VTB (CVC Thermoset Specialties, USA). The following material designations were adopted: F – Flow Art, B – Boston and FM and BM – modified F and B composites, respectively. The measurement of the contact angle Θ and surface free energy (SFE) γ_S was carried out on a DSA30 goniometer (Kruss) using ultra high-quality water (UHQ, PureLab, Vivendi Water) and diiodomethane (Sigma Aldrich) as polar and non-polar liquid, respectively. The liquids were dosed at 4 µL (water) and 1.5 µL (diiodomethane). The smaller volume of diiodomethane was due to the relatively large surface area occupied by the drop concerning the sample surface. The samples were tested 24 hours after polymerization (dry stored) as well as after 24 hours of incubation in distilled water as simulations of the oral environment to evaluate changes in surface properties under the influence of the aqueous environment. Results were statistically analyzed using Statistica software (TIBCO Software Inc.).

Results and Discussion

The values of SEF for the tested materials before and after incubation in water, along with marked statistically significant differences, are presented in FIG. 1. There was a statistically significant reduction in SEP in the case of composite FM compared to F, from the value of 49.33 to 48.42 mJ/m^2. Composite B achieved a higher SEP value compared to F, similar to the BM and FM composites, which indicates the effect of more reinforcement. For composite BM the values were lower than for composite B, however, the differences did not show statistical significance. Incubation in water statistically significantly decreased the values of surface free energy in all cases of the tested materials. Importantly, in all cases of the tested composites, the dispersion component has a decisive share in the size of the surface free energy, which means a higher adhesive affinity for non-polar substances. In the case of the FM composite, the value of this component decreased by 32% compared to the F composite. In the case of the BM composite, an increase in the polar component value was obtained.

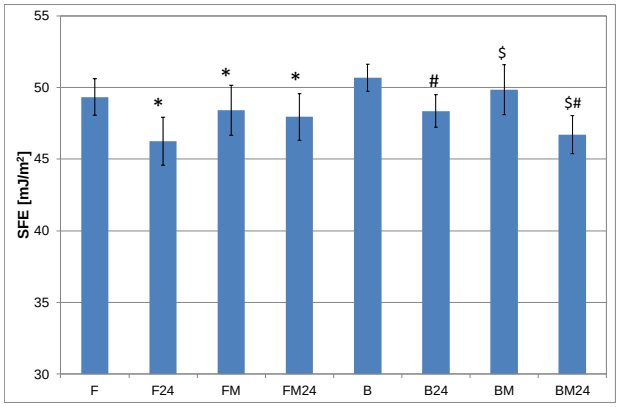

FIG. 1. SFE measurement results for materials immediately after polymerization and after 24 hours of incubation in distilled water. The symbols (*) indicate statistically significant differences against material F, (#) - against material B, $ - statistically significant difference between materials BM and BM24.

Conclusions

Modification of dental composites with liquid rubber favors their hydrophobicity and lowers the value of the surface free energy. It is particularly important in terms of reducing the possibility of colonization of such modified fillings by bacteria

Acknowledgments

The paper was supported by the Ministry of Science and Higher Education in Poland within the statutory activity of Lublin University of Technology (grant FD-20/IM-5/078).

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TIN-FREE BIOELASTOMERIC POLYURETHANES FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 55]

Introduction

DBTDL (dibutyltin dilaurate) is the most popular and effective catalyst which is used for synthesis of biomedical polymers, including poly(lactic acid)(PLA), polyurethanes, and more [1]. However, it is known from the literature that tin compounds show cytotoxicity and have to be removed from the final products. Therefore, the aim of this work is to find an alternative, non-toxic catalyst suitable for the synthesis of telechelic polyurethane macromonomers to create bioelastomers. Several compounds have already been described as highly active in isocyanate groups reaction [2]. Among them, bismuth- and zinc-based catalysts are of great interest, due to their low toxicity and high catalytic activity [3].

Materials and Methods

Synthesis of telechelic macromonomers was performed based on previous work [4] with modifications. Four different catalysts have been used: dibutyltin dilaurate (DBTDL), bismuth neodecanoate (BiNDE), bismuth tris(2-ethylhexanoate) (BiHex), zinc(II) acetyloacetonate (ZnAc). Reactions were performed in two steps. In the first step, 25 ml of ethyl acetate (EtOAc), an appropriate amount of catalyst (2 or 4 mol%) and 6.5 ml (0.052 mmol) of isophorone diisocyanate were added into the flask. Then, 25 g (0.013 mmol) of long-chain diol (Pr, Mn=2000 g/mol) was dissolved in 25 ml of EtOAc and added dropwise into ice-cold mixture. The temperature of the reaction was set to 70°C. Progress of the reaction was controlled by tracking the ratio between FT-IR absorbance at 2262 cm^{-1} and 1526 cm^{-1} which correspond to isocyanate groups and N-H bending vibrations in forming urethane, respectively. In the second step, 6 mg (0.03 mmol) of phenothiazine, the same amount of catalyst as in first step and of 2-hydroxyethyl methacrylate (HEMA) were introduced. Reaction was considered as finished when FT-IR absorbance at 2262 cm^{-1} disappeared. The structure of obtained telechelic macromonomers was characterised with Fourier transform infrared spectroscopy (FTIR, BRUKER ALPHA Platinum apparatus) and nuclear magnetic resonance (NMR, Bruker DPX HD-400 MHz). Obtained liquid macromonomers were photocured with use of photoinitiator 2% w/w, Omnirad 819. 1 mm thick films were produced with use of a steel applicator on glass plate. The composition was irradiated with UV light with maximum intensity at the wavelength λ_{max} of 385 nm in air and argon atmosphere, in a glove box. The intensity of the radiation of the light source (DYMAX Bluewave LED Prime UVA pointer (USA)) was adjusted to 20 mW/cm2 with the help of radiometer, AktiPrint (Technigraf GmbH). The exposure time was 150 seconds for 2.25 cm^2. The cured samples were refluxed for 6 hours by Soxhlet apparatus (Behr Labor-Technik, Germany) to calculate the gel fraction. The cytotoxicity tests were performed according to ISO10993-5 using L929 cell line. Cell viability was then assessed using light microscopy (Delta Optical IB-100, Mińsk Mazowiecki, Poland) and the resazurin viability assay.

Results and Discussion

Chemical structure of obtained macromonomers has been confirmed by ATR-FTIR, ^{1}HNMR and ^{13}CNMR. Cell viability study showed (FIG. 1) that the use of tin-free catalysts, especially bismuth tris(2-ethylhexanoate) (sample PrBiHex_2) and zinc(II) acetyloacetonate (sample PrZnAc_2) resulted in higher cell viability from all tested materials and conditions (catalyst concentration and atmosphere). The differences in cytotoxicity are likely due to the residual amount of DBTDL present in the material. Importantly, we did not observe any effect of atmosphere during photocrosslinking, indicating that the oxygen inhibition is relatively modest.

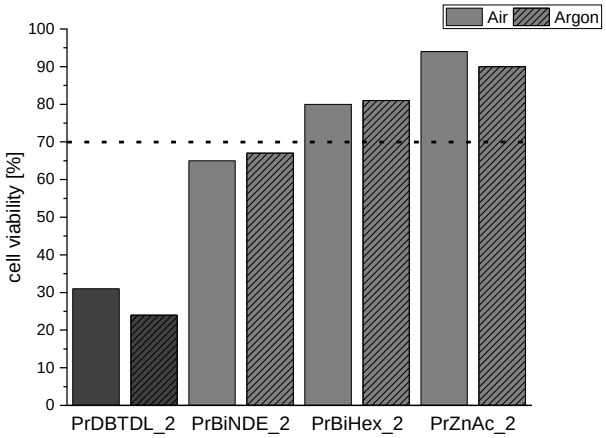

FIG. 1. Cell viability results of materials obtained with 2 mol% of different metallic catalysts.

Conclusions

All catalysts allowed to obtain the same structure of polyurethane macromonomer. Elastomeric networks obtained from macromonomer synthesised with zinc and bismuth catalysts showed lower cytotoxicity as compared to DBTDL. The results indicate high potential of these new materials for medical applications.

Acknowledgments

This work has been supported by research project OPUS17 from the Polish National Science Centre, UMO-2019/33/B/ST5/01445.

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

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[ENGINEERING OF BIOMATERIALS 163 (2021) 56]

Introduction

Biomaterials for bone reconstruction are subjected to continuous improvement [1-3]. However, their properties are still far from expectations, especially in the field of bacterial infections in bone tissue. Antibacterial properties of biomaterials would significantly reduce the problem of infections. The aim of the study was to obtain bioactive and bactericidal glasses for multifunctional composites using the sol-gel method.

Materials and Methods

The developed chemical formulations of bioglass belonged to the ternary 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). As for the composition of ZnO-doped bioglass, 2 wt% (P5Zn2) or 5 wt% (P5Zn5) of CaO in basic glass was replaced with ZnO. Having performed the reaction mixtures from sol to gel and after the drying process was completed heat treatment at 650°C for 15 h The samples were prepared in two grain sizes I and II.
The biological tests were carried out using Human osteoblast cell line hFOB 1.19 in accordance with PN-EN ISO 10993. 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 FIGs 1-3. The cytotoxicity of bioglass containing 5% ZnO exceeded the permissible value of 10%, regardless of the grain size.

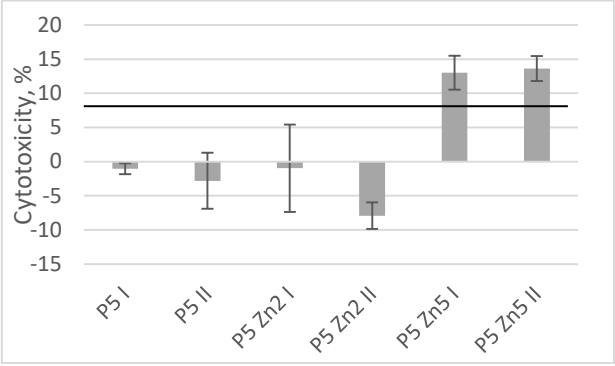

FIG. 1. Bioglass cytotoxicity after 48 h.

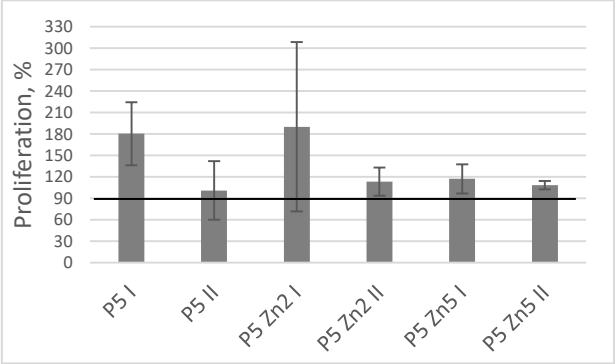

FIG. 2. Proliferation of the hFOB cell line after 48 h of contact with bioglass.

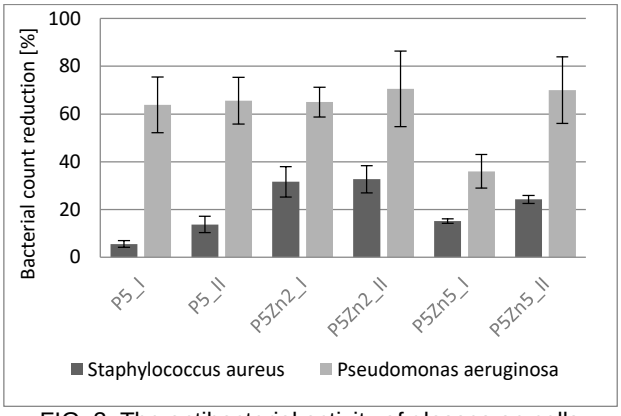

FIG. 3. The antibacterial activity of glasses on cells *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The proliferation of all tested glasses was higher than the required level of 90%. The highest antibacterial effect was demonstrated by bioglass containing 2% ZnO. The level of their antibacterial activity depended on the type of bacterial strain.

Conclusions

The rate of bactericidal reduction of the glasses depended on the ZnO concentration in the bioglass and the susceptibility of the cell culture.

Acknowledgments

Research was funded by the National Centre for Research and Development, Poland (grant no. TECHMATSTRATEG2/406384/7/NCBR/2019)

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CELL ADHESION ON SPIN-COATED POLY(BUTYLENE SUCCINATE-CO-DILINOLENE SUCCINATE) (PBS-DLS) COPOLYESTERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 57]

Introduction

Tissue engineering utilizes polymeric scaffolds to aid in the regeneration or replacement of damaged tissues or organs. In this context, we aimed observe and quantify the interactions between cells and new elastomeric poly(butylene succinate-co-dilinolene succinate) (PBS-DLS) copolyesters, in the form of thin, spin coated films on transparent substrates.

Materials and Methods

The obtained PBS-DLS copolymers were characterized using proton nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). Thin films were prepared from PBS-DLS_CAL-B synthesized with the use of enzyme *C.antarctica* lipase B (CAL-B) [3] and PBS-DLS_C-94 synthesized with the use of titanium oxide/silicon dioxide (C-94) [2], as well as commercial PCL (Sigma, M_n~80kDa) via spin coating on glass microscopy #1.5 coverslips. Samples were examined by Keyence VK9710 laser scanning (LSM) microscope (thickness, roughness) and Krüss drop shape analyzer. Cell adhesion experiments (3 h) were carried out with the use of L929 mouse fibroblasts. Cells were fixed, stained with Hoechst 33342, and imaged using Leica DMi8 inverted fluorescence microscope. Quantification was carried out using Fiji script, available on GitHub (psobolewskiPhD/ImageJ_Macros).

Results and Discussion

Appropriate choice of solvent permitted us to obtain uniform, repoducible thin films ~100 nm thick.

TABLE 1. Characterization of copolymers. Mn: number average molecular weight, Mw: weight-average molecular weight, $L_{BD\text{-}DLS}$: average sequence length, $X_{c,tot}$: total crystalline phase content, R: degree of randomness.

Copolymer	M_w [gmol^{-1}]	M_n [g/mol]	$L_{BD\text{-}DS.}$	$X_{c,tot}$ [%]	R
PBS-DLS 50:50_C-94	50 500	17 400	2.04	35.3	0.83
PBS-DLS 70:30_C-94	171 300	53 500	3.01	40.2	0.87
PBS-DLS 50:50_CAL-B	65 700	18 600	5.07	36.8	0.47
PBS-DLS 70:30_CAL-B	205 600	33 600	6.88	44.9	0.66

TABLE 2. Characteristics of films (n=2-4). Rq: root-mean-square.

Material	Thickness [nm]	Rq_{avg} [µm]	Water Contact Angle (°)	Spherulites [µm]
PCL	95	15	79	6.0
PBS:DLS 50:50_C-94	87	13	86	None
PBS:DLS 70:30_C-94	160	28	87	None
PBS-DLS 50:50_CALB	86	15	89	2.0
PBS-DLS 70:30_CALB	134	25	87	10.0

A clear difference in film morphology was observed between CALB and C-94 materials, with only the former exhibiting spherulites (likewise PCL).

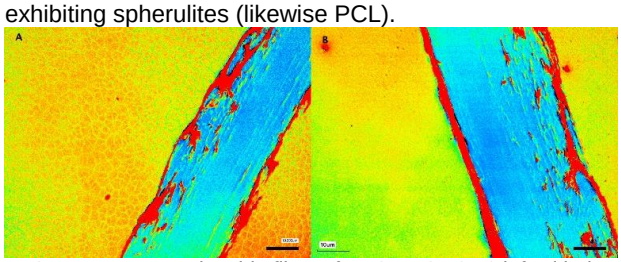

FIG. 1. Representative thin films of PBS-DLS 50:50 left with the use of CAL-B, right with the use of C-94 on coverslips. Scratches were used to measure film thickness.

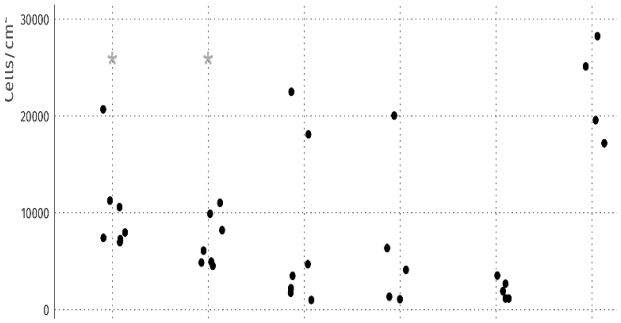

FIG. 2. Attached L929 cells on thin films and tissue culture plastic (TCPS) control after 3 hours. Stars indicate significant difference vs. PCL.

Statistical significance was tested in R software, using Kruskal-Wallis test, followed by Conover's non-parametric many-to-one comparison (two-sided, with Holm p-value adjustment) versus PCL. Post-hoc testing indicates that both PBSDLS5050 materials differed from PCL, which is in good agreement with prior provisional matrix adsorption study [1].

Conclusions

1. Choice of synthesis, enzymatic vs. polycondensation with TiO_2/SiO_2 (C-94) catalyst, influences copolymer block architecture.
2. Enzymatically synthesized copolymers have longer PBS sequences ($L_{BD\text{-}DS}$) than those synthesized using C-94 catalyst which facilitates spherulite formation.
3. Differences in polymer architecture (R, $L_{BD\text{-}DS}$) may explain observed differences in morphology of thin films.
4. Cell adhesion on PBS-DLS with the content of 50 wt% of soft segments was significantly higher than PCL, but no difference was observed between catalysts.

Acknowledgments

This work was financially supported by the National Science Centre, Poland, under the HARMONIA scheme (agreement No. UMO-2014/14/M/ST8/00610).

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THE USE OF A HYDROCOLIDE DRESSING ON A CHRONIC WOUND - CASE STUDY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 58]

Introduction
The treatment of difficult-to-heal wounds with hydrocolloid dressings has been used with great success in human medicine. They are mainly used for exudative wounds, e.g. bedsores, burns and postoperative wounds.
Until now, in veterinary medicine, dressings of this type are also used to treat similar types of wounds. However, animals are more likely to become infected at the site of damage, which significantly hinders their healing and forces veterinarians to perform the general chemiotherapy with antibiotics. The wound heal through granulation for many weeks, leaving a scar and putting a heavy strain on the animal's body. Owners often complain about the length of the therapy.

Materials and Methods
The object of this study was a rat referred by the owners to the Small Mammals Outpatient Clinic at the University of Life Sciences in Lublin with, a ruptured abscess in the thorax area. The lesion was cleaned, general antibiotic therapy and local antibiotic ointment were applied. The animal scratched the wound intensively, which significantly delayed the healing process. Due to the poor condition of the wound, it was decided to use a hydrocolloid dressing sutured in the abscess cavity.

FIG. 1. First day of treatment.

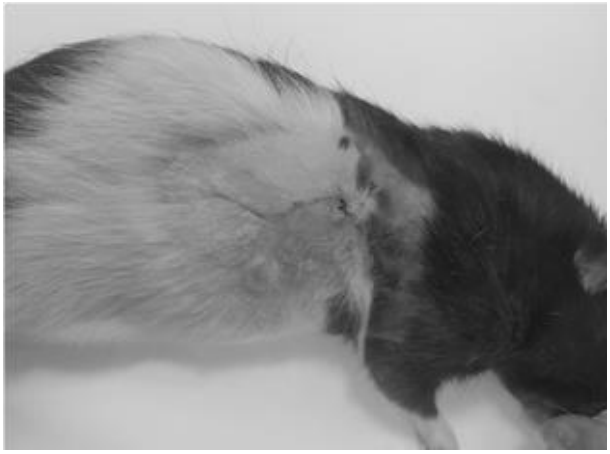

FIG. 2. The wound is healed.

Results and Discussion
After 5 days, the dressing and the scab fell off the healing wound and after 10 days it was healing without any signs of infection.

Conclusions
Hydrogel dressing with the addition of gentamicin works well in the case of chronic difficult to heal wounds. It reduces the possibility of infection, which is common in animal injury cases.

Acknowledgments
The study was supported by National Science Centre (NCN) in Poland within OPUS 16. no. UMO-2018/31/B/ST8/00945 and partly as part of the statutory activities of the UMO (DS3/2020).

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MANUFACTURING AND PROPERTIES OF BIORESORBABLE HYDROPHILIC MICROSPHERES BASED ON POLY[(L-LACTIDE - *CO* - GLYCOLIDE)-*BLOCK*- ETHYLENE OXIDE) AND POLY(BUTYLENE SUCCINATE-*BLOCK*- ETHYLENE OXIDE) COPOLYMERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 59]

Introduction

In recent years, the attention of scientists has focused on the use of natural and synthetic polymers to produce microspheres used in the controlled release systems of various groups of active substances, including drugs.

Therefore, the research aimed to develop the composition and conditions for the synthesis of biodegradable copolymers useful in the formation of microparticles that are carriers of active substances in the processes of their controlled release. It was assumed that the polymer-carriers, obtained based on specially synthesized copolyesters and modified oligosaccharides will be characterized by favorable physicochemical properties allowing for relatively easy formation of microparticles from them, appropriate degradation time, and the release profile of model active substances optimal for the intended use, e.g. in the agrotechnical or pharmaceutical industry [1-4].

The work discusses in detail the method of formation of microspheres and the performance of studies monitoring the course of the hydrolytic and enzymatic degradation of three basic carriers: poly (L-lactide-*co*-glycolide-*block*-poly(ethylene oxide) terpolymers (TER); polymer blends TER/dextrin-*graft*-PCL or TER/maltodextrin-*graft*-PCL and copolymers containing blocks of butylene succinate and poly(ethylene oxide) in the chain (PUR).

Materials and Methods

Materials

Poly(L-lactide-*co*-glycolide)-*block*-poly(ethyleneoxide) terpolymers (Mw = ~28,000 g/mol) were obtained by ROP polymerization of glycolide with L-lactide using PEG 4600g/mol as macroinitiator and $Zr(acac)_4$ as catalyst for this reaction.

The copolymers of dextrin and/or maltodextrin with ε-caprolactone (CL) with various degrees of substitution dextrins with R_{dex} = 70 wt.%. and maltodextrins with R_{dex} = 38 wt.%).

The macroinitiators oligo(butylene succinate) were to be obtained in a modified two-step bulk poly-transesterification of a mixture of methyl succinate with 1,4-butanediol. The obtained polyesters with an average molecular weight of ~5000 g/mol, containing at least two active chain-terminating hydroxyl groups, were used as macroinitiators in the coupling reaction with hexamethylene diisocyanate (HDI) with poly (ethylene oxide). The weight average molecular weight of the copolymer was ~21,000 g/mol.

Methods

Forming the microspheres: The solutions of the polymer mixtures were added dropwise to a small reactor containing a 3% aqueous solution of polyvinyl alcohol (PVA) as an emulsifier. The reactor was connected to a vacuum pump, fitted with a mechanical stirrer, and placed in an oil bath at 23°C. The mixing speed was 120 rpm and the dropwise addition rate was approximately 0.3 ml/min. After the addition of all of the solutions, the temperature was lowered to 18°C while keeping the stirring speed constant. The vacuum pump was then turned on, gradually reducing the pressure to reach full vacuum. The agitation speed was reduced to about 50 rpm and the process was continued under these conditions for 6-10 hours. The obtained microspheres were washed with copious amounts of distilled water and filtered through sieves with a diameter of 150 μm.

Hydrolytic and enzymatic degradation: During the examination of the course of the hydrolytic and enzymatic degradation of microspheres, their specific mass was packed in polypropylene dialysis bags and then each sample prepared in this way was placed in a vial filled with distilled water or activated sludge at room temperature, respectively After the specified time: 0.5; 1; 1.5; 2; 2.5 and 3 months of the experiment, the samples were removed and placed on filter paper and washed in water carefully, but not to damage the samples. The samples were weighed completely after drying.

Results and Discussion

The developed and applied method of forming microspheres allowed for the preparation of microparticles with a relatively regular spherical shape, a fairly smooth and uniform surface, and a relatively narrow diameter distribution with good reproducibility. As expected, with the increase in the degradation time, an increasing weight loss of the samples was observed.

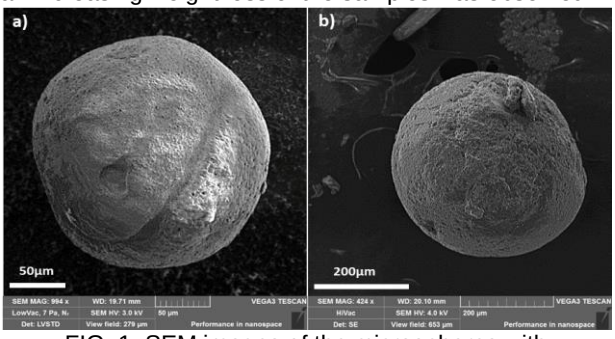

FIG. 1. SEM images of the microspheres with a) Terpolymer, b) copolymers containing blocks of butylene succinate and poly (ethylene oxide) in the chain.

Conclusions

Understanding the mechanism of degradation of these microspheres is a key step in starting research on their use as carriers of active substances in controlled release systems. The obtained microspheres are planned to be used as carriers of active substances, used in the formulation of cosmetics and dermatological preparations.

Acknowledgments

The authors would like to thank the National Science Center, grant no. UMO-2019/33/B/ST5/00743

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BIODEGRADABLE Fe-BASED MATERIALS – A CRITICAL REVIEW

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[ENGINEERING OF BIOMATERIALS 163 (2021) 60]

Introduction

In developed countries, over 20% of deaths are caused by coronary artery disease [1]. To prevent these deaths, is used balloon angioplasty with stent insertion. However, there are still some limitations to the use of stents induced mechanical damage, tissue restenosis, or displacement of the device often necessitate re-operating the patient to remove the stent [2]. The solution to this problem would be bioabsorbable stents, which would fully degrade after a specific time. The most frequently mentioned material for this role are polymers, with polylactide as a pioneer [3]. However, their mechanical properties are poor, so there is a need to use thick walls and stent supports that negatively affect blood vessels' walls and increase the risk of their damage [4]. Therefore, metals that will degrade due to corrosion are increasingly considered suitable materials to create bioabsorbable stents. One of the most frequently mentioned potential biodegradable metals is - apart from zinc and magnesium – iron [5]. It is fully biocompatible and does not cause any toxic effects. Also, its corrosion products are fully biocompatible and tolerated by the human body. Moreover, iron mechanical properties almost perfectly match those of blood vessels. A problem that limits the use of iron as a biodegradable material is its low corrosion rate. It simply degrades too slowly, so there is a need to introduce additional modifications to the surface or structure of the entire material. Additions of other metals are also often used to induce microgalvanic corrosion. [6]. This work takes on this challenge.

Materials and Methods

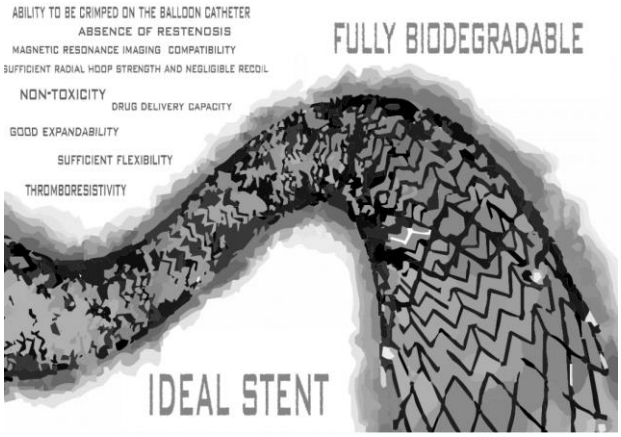
This work uses a straightforward synthesis replica method of nano- or microarchitectural iron and iron-based 3D systems; as templates, were used polyurethane foams and impregnated with the suspension of pure iron. The samples were heated in an oven at a temperature above 80°C, under inert gas conditions, for 8 hours.
The obtained samples were tested by X-ray diffraction (XRD), X-ray spectrometry (EDX), Raman spectroscopy and corrosion analysis in Hank's solution.

Results and Discussion

Research is still in its early stages. However, initial results suggest that it was possible to obtain iron-based materials with different morphology corresponding to the template structure and with a high corrosion rate but with very low mechanical parameters.

Conclusions

The initial results on the corrosion rate of the resulting iron-based materials are very promising as it has been possible to increase the corrosion rate significantly. However, poor mechanical properties prevent the use of this material in the production of a stent. Therefore, there is a need for modification by introducing additional metallic additives or coating the iron with a biodegradable polymer.



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Research financed under the grant NCN Preludium BIS 2019/35/O/ST5/00405.

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PHYSICOCHEMICAL AND *IN VITRO* BIOLOGICAL PROPERTIES OF POROUS PLLA/HAp-Zn COMPOSITES MODIFIED WITH SODIUM ALENDRONATE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 61]

Introduction

Thanks to the appropriate microstructure, porous composite implants, are gaining more and more interest in regenerative medicine for filling bone defects e.g. in osteoporosis. A well-known first-line drug in the treatment of osteoporosis is sodium alendronate from the bisphosphonates (BP) group. The mechanism of action of this drug is based on binding to hydroxyapatites in the bones and inhibiting the activity of osteoclasts, which cause bone tissue resorption [1,2]. However, it does not inhibit the bone formation process and thus causes a gradual increase in bone mass. Unfortunately, BPs show extremely low bioavailability (<1%), poor gastrointestinal absorption and a number of adverse effects when administered orally [3,4]. Hence, there are examples of developing new methods of local delivering of bisphosphonates to the body, e.g. in the form of BP-loaded hydrogels, or drug-eluting implants [5,6].
The aim of this study was to obtain non-cytotoxic, mechanically reinforced, biodegradable, porous composites with a combined osteogenic effect of apatite fillers and sodium alendronate (ALN). Alendronate was introduced into the structure of the porous composite by surface adsorption, and the apatite particles were additionally enriched with bactericidal Zn^{2+} ions.

Materials and Methods

Synthesis of hydroxyapatite whiskers with Zn^{2+} ions (HAp-Zn) was conducted by a homogenous precipitation method with urea with using of aqueous solutions containing sodium dihydrogen phosphate, calcium nitrate tetrahydrate and zinc nitrate hexahydrate (Chempur). The whiskers were characterized by XRD and SEM.
Porous composites were prepared in a thermally induced phase separation process of frozen suspensions of hydroxyapatite whiskers doped by Zn^{2+} ions in 5% solution of polylactide (5%PLA) (PLA-Resomer® LR706, Sigma-Aldrich) in 1,4-dioksane (Avantor). The whiskers content in composites was 25 wt.% (5%PLA25Hap-Zn). The obtained composites were diped for 24 h in sodium alendronate solution (1mg/ml) and dried by lyophilization (5%PLA25Hap-Zn/ALN). The morphology and average pore size of composites was evaluated by SEM. Mechanical properties of the composites were evaluated by compressive test on the Zwick Roell 5kN ProLine test machine for more than 5 specimens at a cross-head speed of 0,6 mm/min.
Cytotoxicity of the obtained composites was determined using standard direct contact cytotoxicity assay with L929 mouse skin fibroblasts, according to ISO10993-5:2009. Analogous tests were performed using human osteoblasts hFOB1.19, a model more relevant to bone implantation. The cytotoxicity assays were complemented using monocyte/macrophage-mediated inflammation sensing system to exclude the activation of human THP1-Blue™ cells in the presence of investigated biomaterials

Results and Discussion

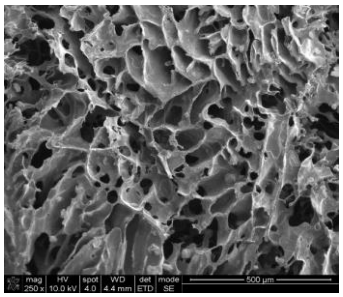

FIG. 1. SEM image of porous composite with the addition of HAp-Zn whiskers.

SEM observations of the obtained composites show that HAp whiskers are embedded in thin walls of the porous scaffolds. Mechanical analysis showed that inclusion of whiskers reinforced the composites. Their compressive strength reaches the level of 0.271±0.057 MPa and 0.373±0.043 MPa for 10% and 20% of strain respectively. The pore size is within the wide range up to 360 μm and can be suitable for the growth of new bone tissue.

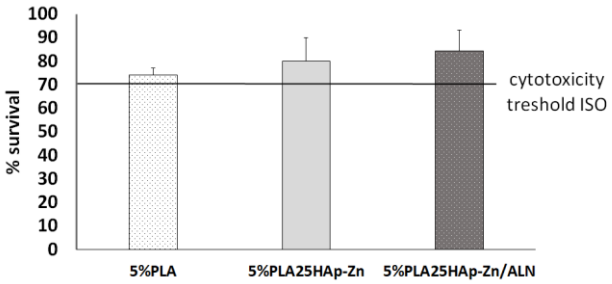

FIG. 2. The results of cytotoxicity assessment according to ISO10993-5:2009.

The effect of direct contact of composites on L929 mouse skin fibroblasts was evaluated in an MTT reduction assay. Percent of survival is shown, in relation to cells grown in the absence of any composite treated as 100%. Percent of survival above 70% is not indicative of a potential cytotoxic effect.

Conclusions

The addition of HAp-Zn whiskers affects the mechanical strength and morphology of porous composites. The surface modification of composite with sodium alendronate allows for a higher % survival of L929 cells.

Acknowledgments

The „Multifunctional composites biologically active for applications in regenerative medicine of bone system" project (POIR.04.04.00-00-16D7/18) is carried out within the TEAM - NET programme of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund.

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COMPOSITE INKS OF HYDROGELS AND INORGANIC BIOACTIVE FILLERS AS POTENTIAL MATERIALS FOR 3D BIOPRINTING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 62]

Introduction

One of the newest technological possibilities for regenerative medicine is 3D bio-printing. This technique makes it possible to print an artificial model of an organ or a scaffold for cells with the use of appropriate components. Typically, bioinks are used for bioprinting, consisting of low-viscosity biocompatible hydrogels. Hydrogels are characterized by a high water content, which provides an appropriate environment similar to the natural extracellular matrix (ECM) for maintaining cell function and viability [1,2]. However, pure hydrogels often exhibit relatively low mechanical and structural stability and often degrade too quickly and unpredictably, which limits their use as bioinks for long-term cell culture or their transfer to in vivo applications [3]. For this reason, composite hydrogel bioinks combining the advantages of hydrogels and various solid fillers are being developed more and more often [4,5].

The aim of the work was to develop new composite inks that would combine the positive features of biocompatible hydrogels with the properties of bioactive inorganic fillers such as bioglass or hydroxyapatite particles. The assumption was that the obtained composite inks were characterized by good printability, and the models obtained after printing had higher mechanical resistance and stiffness than models made of pure hydrogel. The hypothesis was that the use of bioactive fillers with a controlled degree of degradation would allow to obtain inks with the appropriate viscosity, and in the longer term with the appropriate concentration of released ions supporting the adhesion and proliferation of cells in bioinks.

Materials and Methods

Natural polymers were used as the basic components of the hydrogel matrix of composite inks: chitosan and carboxymethylchitosan (Heppe Medical Chitosan). Sodium tripolyphosphate and calcium chloride (Avantor) were used as crosslinkers and stabilizers. As inorganic bioactive fillers the following were used: bioglass with a controlled degree of degradation, enriched with ZnO and nanometric apatite particles.

Bioglass was obtained by the sol-gel method in the SiO_2-P_2O_5-CaO-ZnO system, where the composition was 70 wt.% SiO_2, 5 wt.% P_2O_5, 23 wt.% CaO and 2 wt.% ZnO. After the heating process at 650°C for 15 hours, the obtained bioglass was crushed in a mechanical mortar and ground in a rotary-vibration mill until the grain size was: Dv (0.1) 1.578; Dv (0.5) 7.966; Dv (0.9) 29.258.

The nanoapatite particles were obtained by precipitation from calcium hydroxide and phosphoric acid (Chempur) at pH 11. The composition of the particles obtained was determined by the XRD method as hydroxyapatite.

Composite inks were obtained by mixing the ingredients with the use of two syringes and luer lock adapter. A BIO X, CELLINK printer was used to make 3D prints of the obtained composite inks.

Results and Discussion

Based on selected hydrogels and prepared bioactive fillers, composite inks with solids content of 5-15 wt.% were produced. For the developed inks, printing parameters were established for two types of print mesh for a 3D model in the form of a cuboid with dimensions of 10x10x0.3 mm (FIG. 1).

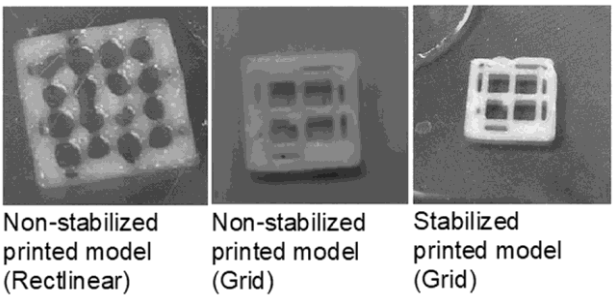

FIG. 1. Images of printouts of composite ink of chitosan hydrogel with 5% of bioglass printed with following print parameters: nozzle diameter= 0,25 or 0,41 mm (25G or 22G), layer height= 0,05-0,1 mm; first layer height= 50-60%; infill density= 15-30%; speed = 2-10 mm/s; p= 10-30 kPa; preflow= 0-10ms

On the basis of the conducted research, it was observed that the addition of inorganic fillers results in higher strength and mechanical stability as well as better reproduction of the shape of the printout. The particle size and the proper distribution of the filler in the hydrogel matrix are important factors influencing the 3D printing process. Too high concentration of inorganic particles greatly increases the viscosity and thus leads to increased pressures during printing. Such an increase in shear forces may have a negative effect on cells in the perspective of future bioprinting, therefore the selection of an appropriate concentration of solid particles is very important.

Conclusions

The developed composite inks based on chitosan and/or chitosan derivatives hydrogels and on selected inorganic bioactive fillers are characterized by good printability and allow for obtaining of stable 3D prints with good shape reflection. The developed methods of their cross-linking and stabilization, the selection of natural hydrogel matrices and the osteogenic properties of the fillers used allow to hope for the use of these composites as potential bioinks for applications in the regeneration of bone or cartilage tissue.

Acknowledgments

Work funded by current operations funding (subvention) from the Ministry of Science and Higher Education for 2021.

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THE DEGRADATION OF BREAST IMPLANTS – IN VIVO AND IN VITRO RESEARCH

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[ENGINEERING OF BIOMATERIALS 163 (2021) 63]

Introduction

Breast implants are used in aesthetic medicine to correct imperfections, as well as in oncology, for reconstruction. Regardless of the main cause of implantation, there are always one reason – a sense of beauty, restoration of lost self – confidence and attractiveness [1]. Unfortunately, each operation carries the risk of complications. The aim of the study was to analyze the degradation of breast implants – in vivo and in vitro.

Materials and Methods

For in vivo studies, samples were taken from implants removed from the body. In vitro tests were carried out in a bacterial solution (Patent nr P 409082) under laboratory conditions. They were dipped for 9 months.
A scanning electron microscope JSM-7800F (FIG. 1) was used to estimate the surface of the implants.

FIG. 1. Scanning electron microscope JSM-7800F.

Bacterial studies were carried out on biological microscope ZEISS Observer D1 (FIG. 2).

FIG. 2. Biological microscope ZEISS Observer D1.

The wettability of the material was tested with a goniometer Theta Life (FIG. 3).

FIG. 3. Theta Life.

Results and Discussion

The structure of the material is irregular with biofilm on it (FIGs. 4 and 5).

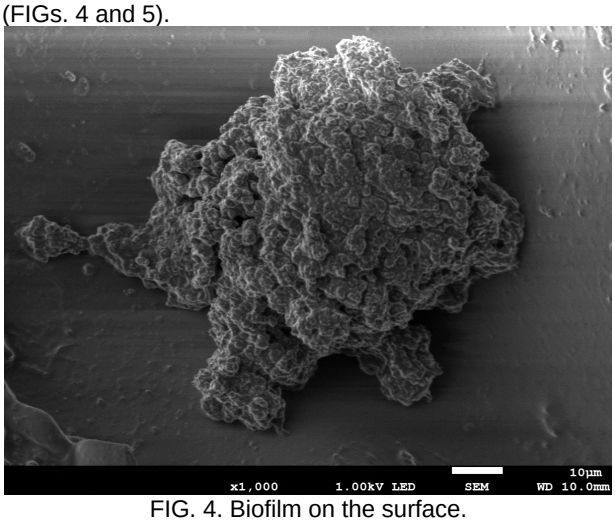

FIG. 4. Biofilm on the surface.

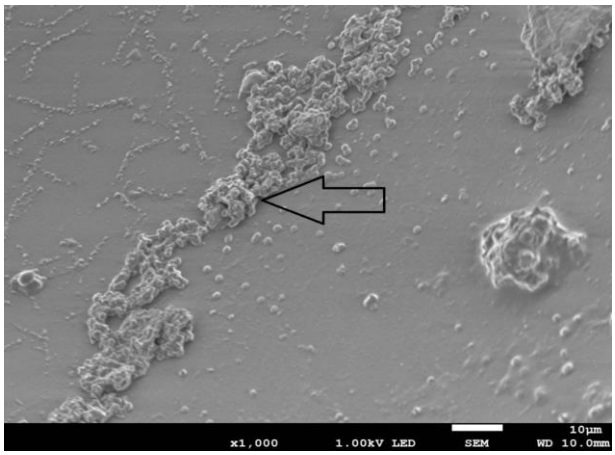

FIG. 5. The chains of bacteria on the surface.

Conclusions

1. The surface of the implants was covered with numerous bacteria and biofilm.
2. The surface wettability tests showed its hydrophobicity.

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TAILORING HYDROPHILICITY OF POLY(BUTYLENE SUCCINATE) (PBS) COPOLYMERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 64]

Introduction

Poly (butylene succinate) (PBS), an aliphatic polyester with excellent mechanical properties and biocompatibility, has recently gained attention for medical applications [1]. Modification of the PBS properties, such as thermal, mechanical and biodegradability rate, can be done by introducing soft segments into the PBS as the co-monomeric units[2]. The copolymers of PBS-dimer linoleic acid (PBS-DLS) and PBS- poly(ethylene glycol) (PBS-PEG) can be found in literature and their tuneable properties was our motivation to introduce those three components in one copolymer structure [3,4]. In this work, the new copolymer of PBS-DLS-PEG with different ratio of the soft segments were synthesized by two step polycondensation. The hypothesis was to take advantage of PEG for improving the surface bio-functionality and wettability at simultaneous good mechanical properties.

Materials and Methods

PBS-DLS-PEG copolymers were synthesized by two step polycondensation. The weight ratio of the hard segments (PBS) was 70 wt.%, while the content of DLS and PEG1000 g/mol and 6000 g/mol, designated as PEG1000 and PEG6000, respectively has been varied from 0% to 30% wt.%. The chemical and mechanical characteristics of the new copolymers were evaluated by Fourier-transform infrared spectroscopy (FTIR), universal testing machine (in dry and wet conditions) and water contact angles. The water contact angle was measured by sessile drop shape analysis according to EN 828 using 2-µL ultra-pure deionized water drop on different areas of the samples. The hydrolytic degradability of the copolymers was also evaluated by immersing the samples into the PBS solution for 18 weeks at 37°C.

Results and Discussion

FIG. 1 represents the FTIR results for the new copolymers with PEG1000 and PEG6000. The results indicated the presence of PEG into the copolymers albeit slight differences can be seen in curves. The main differences can be seen at around 1100 cm^{-1} which is related to C-O stretching of the PEG.

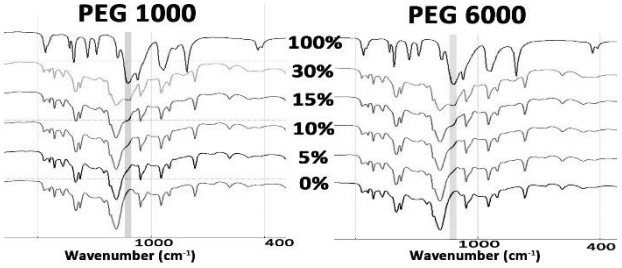

FIG. 1. FTIR spectra of the copolymers with PEG1000 (left) and PEG6000 (right).

FIGs 2 and 3 represent the pictures of the water drop onto the surface of the samples and water contact angles, respectively. The results clearly show the effect of PEG on increasing the wettability of the new copolymers. PEG6000 could make higher impact on wettability compared to PEG1000, that with 15% of PEG6000, the surface properties were varied from hydrophobic (without PEG) to hydrophilic surface.

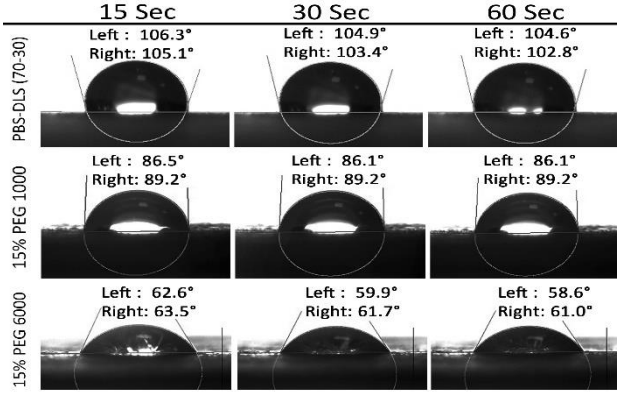

FIG. 2. Micro images of the drop of water onto the surface of the copolymers.

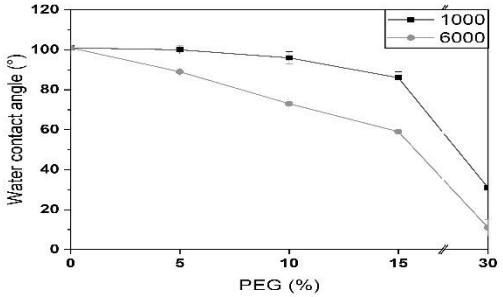

FIG. 3. Changes in water contact angles.

FIG. 4 shows the results from biodegradability after 18 weeks. Addition of PEG1000 slightly increased the biodegradability after week 6th while addition of PEG6000 had higher effect on biodegradability. Since biodegradability has a close relationship with surface hydrophilicity[5], as the presence of PEG600 had higher impact on increasing the wettability, it also had higher impact in increasing the biodegradability.

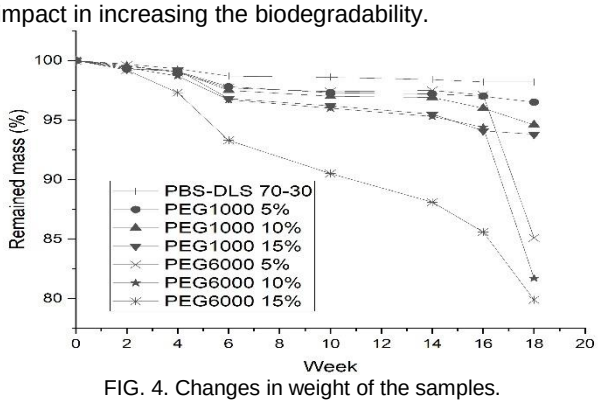

FIG. 4. Changes in weight of the samples.

Conclusions

Hydrophilic PEG1000 and PEG6000 soft segments were successfully introduced to PBS-DLS copolymers as confirmed by FTIR spectra. The water contact angle test showed that incorporation of PEG into copolymers improved hydrophilicity of new materials. Both hydrophobicity and degradation rate of the materials increased as a result of higher content and higher molecular weight of PEG soft segments.

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SURFACE MODIFICATION OF CARDIAC STENTS USING NEW COORDINATION COMPOUNDS WITH POTENTIAL ANTITHROMBOGENIC PROPERTIES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 65]

Introduction

The safety and efficacy of percutaneous coronary intervention have improved significantly over the past 40 years. In particular, the introduction of a drug eluting stent (DES) was an important breakthrough in interventional cardiology [1,2]. Currently, newer and newer drug-eluting stents are appearing and further clinical trials are being conducted to assess their efficacy and safety [3]. The disadvantage of DES stents is the increased likelihood of late thrombosis on compared to BMS. This is due to the fact that DES release antiemotic drugs (sirolimus, paclitaxel, everolimus or zotarolimus), which delay the endothelium of the stent surfaces. Patients requiring DES implantation must therefore receive PCI antiplatelet therapy. [4,5]. Drug-eluting stents (DES) have three components: a metallic stent platform, a polymer coating, and a medicinal agent (FIG. 1) [6,7]. Polymer coatings that are applied to the surface of the stent serve as drug carriers and allow controlled drug release. Advances in polymer technology aim to reduce local inflammatory reactions and thrombosis by improving the biocompatibility of polymers. Currently, biodegradable polymers are being introduced, and during their decomposition, the drug is gradually released [7]. Our research will concern the modification of stent surfaces with a bioresorbable polymer, which will gradually release the anticoagulant drug.

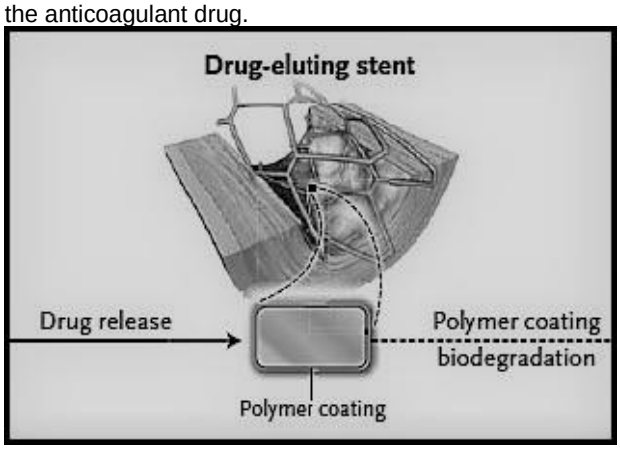

FIG. 1. Construction of drug-eluting stents [5].

Materials and Methods

A key point in our research is the synthesis of coordination compounds with the desired antithrombogenic properties. New coordination compounds were obtained through the synthesis of titanium(IV) alkoxides with substance possesing anticoagulant properties (acetylsalicylic acid (ASA)). It is also planned to obtain titanium (IV)oxo-complexes with other anticoagulants such as warfarin, clopidogrel and heparin.

The reaction of Ti(IV) alkoxides with acetylsalicylic acid was carried out in a 4:1 molar ratio. For this purpose, 0.16 g of ASA was dissolved in 1 ml of a solvent (THF/ BuiOH (1:1), THF/PriOH (1:1)) and added to 1 ml of titanium (IV)isopropoxide or 1.19 g of titanium(IV) isobutoxide, in room tempaerature, under Ar.

Results and Discussion

The obtained results of the synthesis of titanium (IV) oxo-complexes with acetylsalicylic acid seem to be promising. We managed to obtain a crystalline form necessary for detailed structural studies of the compounds obtained.
Reactions conducted with titanium (IV)isobutoxide seem to give better results than using titanium(IV) isopropoxide. Out of the various solvents tested, the 1: 1 mixture of THF with BuiOH seems to be the best.

Conclusions

The conducted research proved that it is possible to synthesize titanium (IV)oxo-complexes with such anticoagulant, as ASA, using the method proposed by our team. Further studies will be carried out with the use of other anticoagulants (warfarin, heparin, clopidogrel). Studies show [8] that combination therapy and dual antiplatelet therapy with aspirin and clopidogrel and warfarin are also possible. This knowledge can also be an inspiration for our further research.

Acknowledgments

Authors would like to acknowledge Nicolaus Copernicus University in Torun for the financial support of research within “DEBIUTY” grant.

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THE BIOLOGICAL DEGRADATION OF LCP PLATES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 66]

Introduction

Nowadays, the most frequently used method of fracture fixation in orthopedics is internal fixation with the use of LCP (Low Contact Plates) plates. They are connected to the bone in multiple points – with the use of screws. That`s why they differ from those used previously. The LCP plates have a complex geometry and are use for fractures in places requiring precise reconstruction [1].
The paper presents the biological degradation of LCP plates in vivo. Microorganisms are able to damage the structure and function of implants, especially when they are in contact with a living organism, the most aggressive environment.

Materials and Methods

The material was removed from the body due to infection. Four plates LCP (FIG. 1) with visible changes on the surface were analyzed.

FIG. 1. LCP plates.

A scanning electron microscope JSM-7800F (FIG. 2) was used to estimate the surface of the plates.

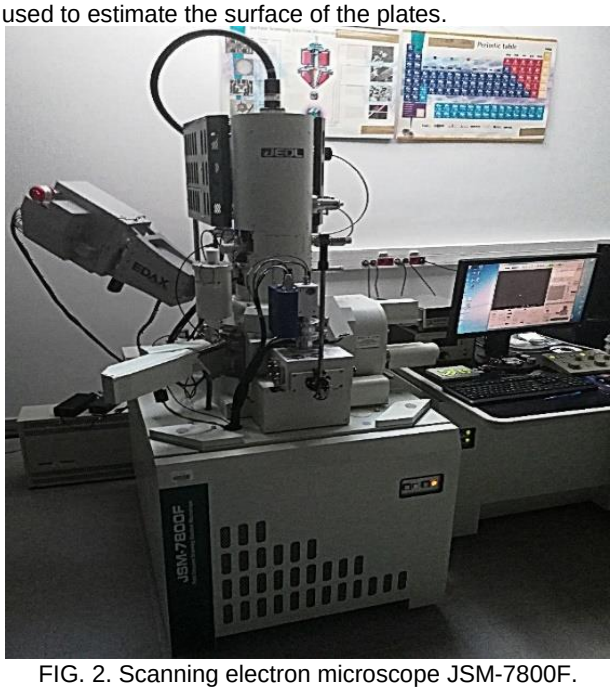

FIG. 2. Scanning electron microscope JSM-7800F.

Bacterial studies were carried out on biological microscope ZEISS Observer D1 (FIG. 3).

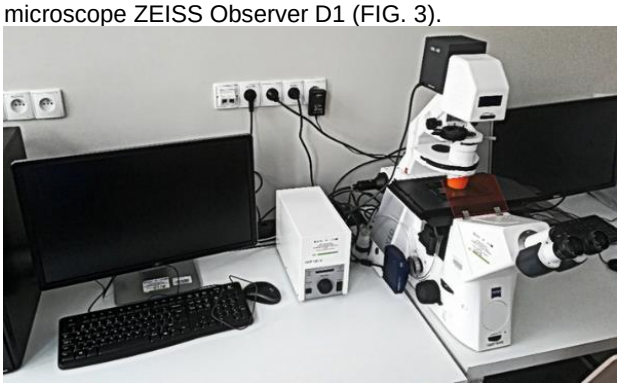

FIG. 3. Biological microscope ZEISS Observer D1.

Results and Discussion

After removing the plates from the body, the plaques were observed on the surface (FIG. 4).

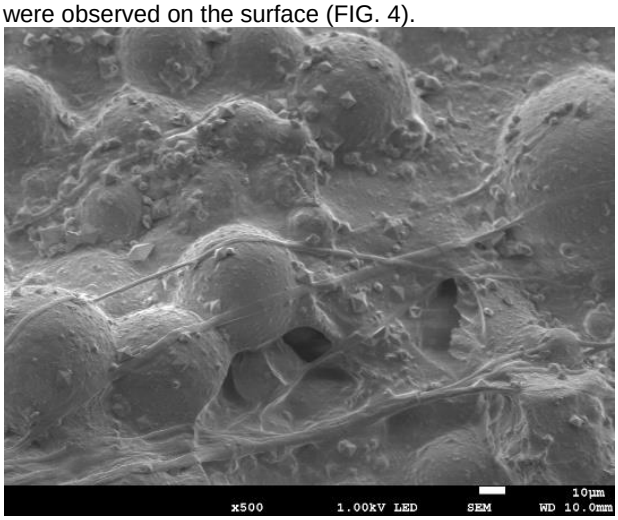

FIG. 4. The plaques on the surface. Scanning electron microscope.

The plaques was observed on the biological microscope. It was a well-developed biofilm (FIG. 5).

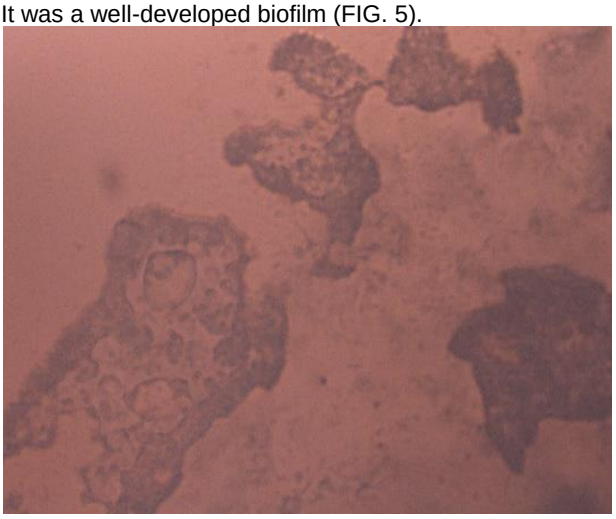

FIG. 5. Biofilm. Biological microscope ZEISS

Conclusions

1. The plates were covered with biofilm.
2. Further studies showed, that the bacteria had penetrated the material causing pitting.

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INVESTIGATIONS OF RHEOLOGICAL AND TRIBOLOGICAL PROPERTIES OF SOME SYNOVIAL FLUIDS SUBSTITUTES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 67]

Summary

Ensuring adequate lubrication in the synovial joint is extremely important for the proper functioning of the locomotor system. Low movement resistance and favorable viscoelastic characteristics are features that define a healthy joint. Natural biomolecules – present in the synovial fluid – are responsible for the lubrication of the articular surfaces. When they are degraded, due to disease or injury, the joint becomes dysfunctional. In such cases, the most common medical procedure is the administration of a series of injections in order to deliver the biolubricant into the joint's space. The preparations that are substitutes for synovial fluid should be characterized by high biocompatibility as well as the appropriate rheological and tribological properties.

In this paper, the basic compositions of the artificial synovial fluid were tested. They consisted of low-molecular polyacrylamide, high-molecular polyacrylamide and hyaluronic acid sodium salt. For the comparative purposes, the commercial preparation named Hyalgan, which is used in viscosupplementation treatments, was also tested. During the experimental research, physicochemical measurements were carried out, such as the measurement of the pH value, electrolytic conductivity and surface tension. In order to determine the properties of the viscoelastic substitutes, rheological tests were carried out with the use of a rotational rheometer. To determine the resistance to motion, friction tests were carried out for reciprocating motion. Additionally, energy dissipation was estimated, and microscopic analysis of friction traces was performed. This gave a possibility to determine the volume of friction traces and to compare the influence of the tested lubricants on the wear intensity of the samples.

The obtained test results indicate that preparations based on polyacrylamide have a beneficial effect on lowering the resistance to motion in the tested tribological systems. They are also characterized by advantageous viscoelastic properties. It is worth noting that among all the tested synthetic synovial liquid compositions, the preparation based on high molecular weight polyacrylamide showed the most promising functional properties.

STEM CELLS IN DIABETES – WILL IMPLEMENTING INTERDISCIPLINARY ONLINE COLLABORATION HELP TO DISPEL ETHICAL RESISTANCE?

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[ENGINEERING OF BIOMATERIALS 163 (2021) 68]

Introduction

According to the World Health Organization's statistics published in 2018 [1], the number of people with diabetes has increased from 108 million in 1980 to 422 million in 2014 and still growing [1]. Only in Poland the number of deaths attributable to high blood glucose in 2016 was estimated at 25 800 [1]. International Diabetes Federation estimates that by the year 2045 the number will reach 629 million [2]. There used to be only 3 types of diabetes, nowadays, 12 different types of diabetes have been distinguished during the past 20 years [3]. Rapid changes and general misinformation may lead to lack of trust and ethical resistance towards new treatment methods.

Materials and Methods

Cell-based therapies for beta-cell replacement are now under intensified investigation. Researchers have been advancing methods to generate insulin-producing beta cells from pluripotent stem cells (PSC) for the clinical treatment of diabetes [4]. However, apart from the stem cell ethical factors [5], physicians and scientists have more moral dilemmas connected with the selection of patients for the treatment and possible risks. Mostly mentioned risks are tumors, the growth of the stem cells into unwanted cell types and taking immunosuppressive drugs that suppress the activity of the immune system. It is also difficult to regulate how much insulin the new beta cells produce [4-7]. Patients' physical and psychological reaction to that treatment could be difficult to predict. Without good collaboration and support stem cell treatment may also become harmful to the good habits worked over the years. In order to monitor patients physical and emotional well being a multidisciplinary collaboration would be set up and carried out through the whole preparation and treatment process, and also afterwards. Establishing cooperation between specific groups of interdisciplinary specialists, such as engineers and physicians, has a significant impact on modern diagnostics and medical treatment development [8-9]. Interdisciplinary med-tech projects have been carried out online before thanks to Moodle for Teachers, ERASMUS+ SP4CE project and keen collaboration between universities and entrepreneurs. One of the projects, carried out fully online and successfully completed was an individual CT-based mandible implant design created by two students from Gdańsk University of Technology, working with students from Medical University of Gdańsk. [8-9].

Results and Discussion

Using stem cells in diabetes should as an attempt to cure the disease brings up many heated discussions. The objective to work on this method is to save patients from having to monitor their glucose level many times a day and release them from insulin injections. However, even if the stem cells method proves to be effective, patients will have to be condemned to immunosuppressive drugs that may cause many side effects, which may lead to the need of additional medical and emotional support [7-8]. Establishing a collaboration platform for bioengineers, physicians, therapists and other necessary specialists could bring many benefits and cause a serious impact in sharing knowledge and discussing individual case studies, which results in faster and more effective development of stem cell treatment, as well as the increase of knowledge about side effects and risks that could be impossible to predict in the pre-treatment studies [8-9].

Conclusions

Stem cells treatment should be provided with a multidisciplinary approach, including the proper collaboration and consistency of the study. Patients undergoing experimental therapy must be provisioned with well-organized during-and-after-care consisting of multidisciplinary specialists. They should be able to receive the necessary support from general practitioners, nurses, physiotherapists, psychotherapists, and social workers. LMS Moodle with the variety of tools allows for many adaptations of the working area that supports online collaboration in multiple ways in order to meet the needs of innovative treatment in diverse disciplines.

Acknowledgments

Although ERASMUS+ SP4CE project was funded by the European Commission, EU is not responsible for the content of the publication.

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DEPOSITION OF IRON ON CARBON NONWOVENS BY MAGNETRON SPUTTERING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 69]

Introduction

Cavities in cartilage and bone tissue are outcome of diverse diseases, accidents or tumors. Larger defects must be adequately supplemented by transplantation method using a wide variety of implants including synthetic or natural allo-, auto- and xeno- geneic materials. One of the different approach to treatment is usage of materials forming a tissue scaffold. The ideal scaffold has some particular requirements such as biocompatibility in both primary and degraded form along with uniform porosity that allow access of nutrients to the treated tissue. Ideally, the outer layer of such material should exhibit structural and surface compatibility with processes of bone tissue cell adhesion and proliferation [1]. A modern approach in biomaterials engineering is utilization of carbon fibers, using polyacrylonitrile (PAN) fibers as a precursor, as a tissue scaffold. For this reason, proper selection of precursor fibers is crucial, since strength and porosity of the carbon fibers depend as well as on the type of precursor. The aim of the study was to investigate the possibility of obtaining carbon nonwovens from needle punched, precursor PAN nonwovens. Prepared in such way nonwovens may become a scaffold for bone tissue regeneration.

Materials and Methods

The study was conducted on PAN needle punched nonwovens, prepared from fibers 60mm in length, previously oxidized. Several variants of nonwovens were produced, with different surface mass, i.e. 80 g/m^2 (PAN1), 120 g/m^2 (PAN2), 190 g/m^2 (PAN3), 330 g/m^2 (PAN4), 600 g/m^2 (PAN5). Determination of parameters such as: tensile strength (PN-EN 29073-3:1994), thickness (PN-EN ISO 9073-2:2002), swelling and absorbency at free soaking in NaCl and PBS, the presence of chloride ions and heavy metals have been conducted. Precursor nonwovens were subjected to carbonization process, using a two-stage thermal treatment process. Iron was deposited in the obtained carbon nonwovens using the magnetron sputtering method on a DC magnetron manufactured by P.P.H. Jolex s.c. (Częstochowa). The modification was carried out in argon atmosphere (4%) for 10 min, at effective power 1 kW and working pressure 2.9x10^{-3} mbar.
After iron deposition, the morphology of the fibers surface was examined the use of Tescan Vega 3 scanning electron microscope (Tescan Analytics, Brno, Czech Republic) equipped with the EDS (Oxford Instruments, Abingdon, UK) X-ray micro analyser.

Results and Discussion

Results analysis as well as preliminary carbonization tests revealed that the most optimal structure and properties (based on the declared and intended use of such material) have been observed for samples with lower surface mass. Such nonwovens were characterized with the highest air permeability parameter, which may be beneficial. For samples with higher surface mass, the inefficient flow of thermal energy to inside of the nonwoven was observed, what very likely disturb natural body thermoregulation. Therefore, for further tests sample PAN2 (120 g/m^2) has been selected.
Analysis of the swelling results - fluid handling capacity and dehydration rate demonstrated that the PAN2 nonwoven showed no significant swelling ability in both fluids, NaCl and PBS. Fluid handling capacity and absorbency tests presented similar characteristics for both fluids. SEM+EDS analysis indicated the regular distribution of iron on the fiber surface (FIG. 1).

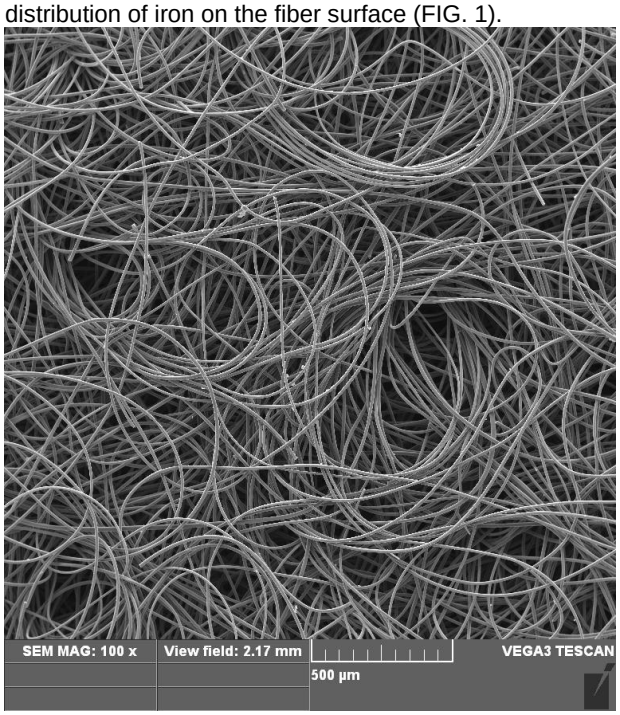

FIG. 1. SEM image of iron modified carbon nonwovens.

Conclusions

The analysis of the research showed that the PAN2 nonwoven is characterized by the most optimal structure and properties, based on declared and intended use of such material. Nonwovens obtained as shown ensure homogeneous supply of thermal energy to the entire volume of the material. Additionally, regular distribution of iron and uniform metallic surface into a polymer nonwoven material exhibits a potential for its imaging after implantation, e.g. using magnetic resonance.

Acknowledgments

This research was funded by the National Science Centre, Poland, grant number UMO-2018/31/B/ST8/02418.

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STUDIES OF THE INFLUENCE OF SURFACE TOPOGRAPHY OF TI6AL7NB ALLOY ETCHED IN CF_4 PLASMA ON ITS SELECTED PROPERTIES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 70]

Introduction

The subject of surface patterning of titanium alloys has been attracting a large interest among scientists for decades. However, so far, these materials have been surface structured mainly by laser ablation method, which allows to obtain a variety of shapes with an anisotropic profile. According to numerous literature reports, laser surface texturing affects wettability [1-3], improves mechanical properties and wear resistance [4], as well as biological properties, e.g. faster development and spreading osteogenic cells [5-7] or increasing antibacterial properties [8]. Plasma etching process seems to be a very promising method of patterning of titanium alloys surface and good alternative for laser structuring techniques. The abovementioned method has been widely studied for the last decades in the field of fabrication of silicon structures for microelectronics applications. In comparison with laser texturing method, a wider range of profile shapes could be obtained, from isotropic to anisotropic one. The etching directivity is determined by the dominant etching mechanism - chemical or physical, depending on numerous factors, such as e.g. process parameters, chemical composition of reactive gas, substrate and mask material and its geometry. The surface treatment of a silicon substrate using fluorine-based plasma leads to obtain mainly isotropic profiles [9]. Furthermore, the presence of CF_x radicals may cause a competitive process - formation of a fluorocarbon film [9]. So far, the knowledge in the field of etching mechanisms (including CF_4 plasma etching) of titanium alloys is insufficient and studies in this area require to be expanded. In this study, the high potential of the CF_4 plasma etching connected mainly with the possibilities of obtaining the different shapes of the etched profiles became the motivation for the modification of the Ti6Al7Nb alloy surface using this method.

Materials and Methods

In this study, CF_4 plasma etching processes on mechanically polished surface of the Ti6Al7Nb alloy were carried out. The surface topography was shaped using AISI 316L stainless steel masks of different geometry and different process parameters. Two values of self-bias (-500V, -700V) and pressure (0.65 Pa, 1.30 Pa) were applied. The process time (60 min) and gas flow rate (10 sccm) remained the same for all the conducted etching processes. The surface morphology was analysed by optical microscopy (Keyence VHX) and scanning electron microscopy (Jeol JSM – 6610L). The measurements of the etched structures and masks after the etching processes using the images captured through the optical microscope were also performed. The chemical composition of the patterned Ti6Al7Nb alloy surfaces was investigated using energy dispersive spectroscopy (EDS). The contact angle measurements were carried out using the sessile drop technique and KrussEasy Drop FM40 apparatus. Surface roughness was measured using the stylus profilometer (Hommel Tester T1000) in accordance with PN-EN ISO 4287. Using this method, the depth measurements of the etched structures were also carried out. Based on the dimensions of the etched structures and masks used during the etching processes, the etching directionality and selectivity were calculated.

Results and Discussion

Scanning electron microscopy (SEM) has shown the presence of the reaction products on the modified surfaces, especially located under the mask material. EDS microanalysis has confirmed the presence of elements derived from CF_4 plasma (C, F) and the mask material (Fe, Cr, Ni). The studies on the etching mechanisms have exhibited strongly isotropic profiles for the lowest self–bias value (-500V). The application of a higher negative self–bias value resulted in the action of both free radicals and ions, thus showing a mixed (chemical and physical) nature of the etching mechanisms. The highest selectivity was obtained applying -500V self-bias value and pressure of 0.65 Pa, probably due to the lower ions energy resulting in less effective mask material sputtering. The surface roughness of the etched titanium alloy has increased after CF_4 plasma modification. Vertical roughness parameters (R_a, R_z) have achieved the lowest values at -500V self – bias value. Surface roughness defined by R_{Sm} parameter depends on the geometry of the etched pattern. The contact angle has increased from approximately 70º for polished Ti6Al7Nb sample to 90º for the patterned one, thus increasing the hydrophobic properties of the modified surface.

Conclusions

Process parameters, especially self – bias and pressure values, have a significant influence on etching directivity and dominant etching mechanism, resulting in obtained profiles from strongly isotropic towards anisotropic one. The EDS microanalysis confirmed the presence of carbon and fluorine atoms on the patterned surface in CF_4 plasma, what can be related to a formation of a thin fluorocarbon film. The change in the wettability of the surface results from an increase in surface roughness and a change in the chemical composition after the plasma etching processes.

Acknowledgments

The studies were financially supported from the project no. 2018/31/D/ST5/03581/ Badania mechanizmów trawienia powierzchni stopu tytanu Ti6Al7Nb w plazmie na bazie fluoru funded by The National Science Centre (NCN).

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THE ETCHING PROCESS OF THE SURFACE OF Ti6Al7Nb ALLOY IN SF_6 PLASMA AND ITS INFLUENCE ON THE SELECTED PROPERTIES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 71]

Introduction

In the field of surface engineering, continuous development is required in terms of technologies allowing for modifications of the materials surfaces that enable to obtain the most favorable properties for selected applications. Modification of the material surface affects its morphology and topography, at the same time changing specific properties. A well-known and most commonly used titanium alloy surface treatment is laser modification [1-3]. However, with a step forward, attention should be focused on the development of a promising and easy to implement in the industry method which is plasma etching, that achieves the intended topography of the titanium alloy surface. In the plasma etching process two fundamental etching mechanisms can be distinguished: chemical and physical one. In the case of the chemical mechanism the active species (radicals) originating from plasma react with the material of the substrate leading to the formation of stable volatile products, and as a result in the removal of a certain amount of matter. Whereas in the case of the physical mechanisms high-energy ions play an important role, removing particles from the modified surface. Depending on the chemical composition of plasma, process parameters and the substrate material, the obtained etching profiles show anisotropic or isotropic character [4,5]. The SF_6-based plasma etching processes promote a chemical etching without any noticeable coating of the surface with fluorine molecules. Moreover, studies show that increasing the amount of fluorine containing molecules increases the etching rate, but also affects the etching result of a more isotropic profile [6]. Currently, despite great potential of plasma surface treatment, there is a lack of studies in the world literature concerning the influence of the etching process parameters (i.e., pressure, bias, substrate temperature) in SF_6-based plasma on surface properties of titanium alloy etched using a mask of appropriate geometry. Therefore, in this study, the aforementioned method became the motivation for the modification of the Ti6Al7Nb alloy surface in the SF_6-plasma.

Materials and Methods

Samples of Ti6Al7Nb alloy with mechanically polished surface were chosen as the substrates for the study. Plasma etching processes were carried out using SF_6 gas. The surface topography was shaped using AISI 304L steel masks. The effects of various process parameters on the surface morphology and topography were investigated. Two different values of self-bias (-500V, -700V), pressure (0.65 Pa, 1.3 Pa) and substrate temperature (cooled, heated up to 230℃) were applied. The process time (30 min) and gas flow rate (10 sccm) remained the same for all the etching processes carried out.

The surface morphology and chemical composition of the modified surfaces were examined with the use of JSM-6610LV (JEOL) scanning electron microscope (SEM) integrated with the EDS X-MAX 80 analyzer (Oxford Instruments). The surface morphology was also studied by optical microscopy (Keyence VHX). The measurements of the etched structures and masks after the etching processes using the images captured through the optical microscope were performed. Surface roughness was measured and analysed using the contact profilometer (Hommel Tester T1000) and EVOVIS software in accordance with PN-EN ISO 4287. The depth measurements of the etched structures were also carried out. The etching directionality and selectivity were calculated.

Results and Discussion

SEM analysis of the samples etched in SF_6 plasma revealed the presence of by products on their surface. Microanalysis of the modified surfaces using energy dispersive spectroscopy (EDS) showed the presence of fluorine derived from SF_6 plasma and elements from the masks material (Fe, Ni, Cr). The higher concentration of fluorine was observed on the surface of the heated samples compared to the cooled substrates. The roughness parameters measurements of the etched surfaces (Ra, Rz) revealed the decrease in surface roughness for cooled samples with the decrease pressure value. The lowest etch selectivity was obtained at -700V self-bias value and pressure of 1.3 Pa due to higher ions energy resulting in sputtering of the mask material. The highest anisotropy was obtained for the cooled sample etched at a potential of -500 V and a pressure of 0.65 Pa.

Conclusions

This work deals with the SF_6 plasma etching of the surface of Ti6Al7Nb alloy. The changes in the chemical composition, morphology, roughness, etch directionality and selectivity were analysed depending on the pressure, bias and substrate temperature values applied during the etching process. It has been proven that higher substrate temperature increases the surface roughness. For etching processes in SF_6 plasma, the surface roughness increase with increasing bias values. The high value of the bias results in a decrease of the etch selectivity.

Acknowledgments

The studies were financially supported from the project no. 2018/31/D/ST5/03581, entitled: Badania mechanizmów trawienia powierzchni stopu tytanu Ti6Al7Nb w plazmie na bazie fluoru, funded by The National Science Centre (NCN).

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DEVELOPMENT OF BIORESORBABLE MONOFILAMENT INCREASING THE FUNCTIONALITY OF 3D PRINTING TECHNOLOGY IN BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 72]

Introduction

The great advantage of 3D printing in the field of the biomedicine is the ability to create personalized implants or surgical phantoms with the support of computed tomography or magnetic resonance imaging [1-2]. The increase in the use of this technology has also caused the development of the filament market, but the availability of certified filaments, especially for biomedical applications, is still insufficient. The study aims to develop a filament based on poly(lactide-co-glycolide) (PLGA) copolyester on the potential application of obtaining scaffolds by 3D printing.

Materials and Methods

Poly(L-lactide-co-glycolide) copolyester with molar composition of 85:15 mol % of lactidyl to glycolidyl units (PLGA 85/15), was obtained via ring-opening polymerization (ROP) of cyclic monomers: L-lactide (HUIZHOU Foryou Medical Devices, China) and glycolide (HUIZHOU Foryou Medical Devices, China) in the presence of a zirconium (IV) acetylacetonate $Zr(acac)_4$ initiator (Merck, Germany) in an amount of 1:3000 on a molar ratio to the monomer mixture [3]. The synthesis was carried out in a Teflon reactor, as well as, attempts have also been made to scale-up the production using a mixer device (30EHT 3Z, Brabender, Germany).

The 3D printing filament was produced using a twin screw extruder (TSE 20/40, Brabender, Germany). Process was carried out at a temperature of 170°C. The filament with a diameter of 1.75 ± 0.05 mm was produced and used for the preparation of polymer scaffolds.

3D printing was carried out via FDM (fused deposition modeling) method using the Flashforge Dreamer dual extrusion 3D printer (Flashforge Corporation, China). The influence of the infill configuration of the individual scaffold layers on the effectiveness of the colonization by fibroblasts was tested.

The molar composition of the copolyester as well as the progress of the polymerization reaction was characterized by nuclear magnetic resonance (^{1}H NMR) spectroscopy (600 MHz Bruker Avance II Ultrashield Plus, USA). The average molar mass was determined by gel permeation chromatography (GPC) (Spectra Physics SP 8800 chromatograph, USA). Thermal properties were investigated by differential scanning calorimetry (DSC) (Q2000 DSC, TA Instruments, USA). The fibroblast culture was observed using a confocal microscope (LSM 710 Zeiss, Carl Zeiss Microscopy GmGB, Germany).

Results and Discussion

It was observed that conducting the ROP reaction with the use of the mixer, thanks to the better mass and energy exchange within the reaction chamber, allows for a significant shortening of the polymerization reaction time. However, the polymer material obtained in this way had a slightly lower number average molar mass (Mn) but the dispersity was comparable.

Optimization of the 3D printing process was carried out in terms of obtaining scaffolds with a single layer component resolution equal to 200 μm and 200 um of height and width respectively. Printing parameters were chosen to obtain a continuous stream of molten PLGA 85/15 polymer, which allowed obtaining a good-quality openwork structure of the scaffold with repeatable pore sizes. Quality control was performed using a stereoscopic optical microscope (IPOS 810, Delta Optical, Poland). For the in vitro experiment, scaffolds with a single layer infill density of 40%, differing in the way of printing: triangular and grid patterns, were selected. In vivo fibroblast culture observation was carried out for 8 days FIG. 1.

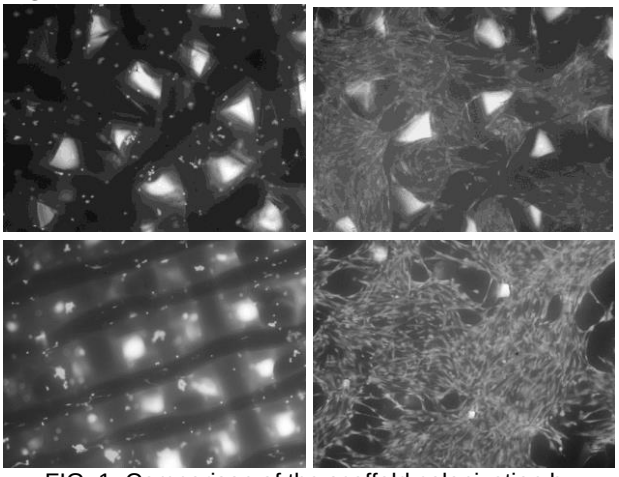

FIG. 1. Comparison of the scaffold colonization by fibroblasts after 2 days (left) and 8 days (right). Infill pattern type: triangular (top) and grid (bottom).

Conclusions

The PLGA 85/15 filament, produced by industrially available methods, meets the requirements allowing it to be used for the production of scaffolds using FDM 3D printing. It has been proven that it is possible to culture fibroblasts. The colonization of the scaffold by cells depends on the size and shape of pores, which can be controlled by the 3D printing method.

By 2023, it is planned to introduce to the market Polish PLGA medical grade filament.

Acknowledgments

Acknowledgements to Dr Tomasz Cichoń and Dr Ryszard Smolarczyk from the Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice Branch, Poland for sharing the images obtained with the confocal microscope.

A financial support has been provided by POIR.01.01.01-00-2116/20-00, acronym 4Medprint.

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THE TECHNOLOGY FOR DEVELOPING AND OBTAINING THE NEXT GENERATION OF VASCULAR STENTS THROUGH MICROINJECTION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 73]

Introduction

Cardiovascular diseases (CVD) are currently one of the most important problems in medicine. According to the World Health Organization, diseases of the cardiovascular system are the greatest cause of death for people all over the world. WHO estimated 17.9 million people died from CVDs in 2019, accounting for 32% of all deaths worldwide [1]. Low-invasive procedures, including coronary angioplasty, are among the most promising and effective methods of preventing the effects of coronary heart disease and cardiovascular diseases. Bioresorbable vascular stents (BRS) pose an interesting alternative for metal vascular stent, because they provide mechanical support and are not burdened with typical disadvantages resulting from the use of metal stents, such as vascular inflammation and thrombosis [2,3]. Despite processing difficulties, bioresorbable polymer materials are the subject of intensive scientific research and found wide application in medicine and pharmacy due to their unique properties [4].
The aim of the research is to develop technology for the production of a new generation of biodegradable vascular stents by micro injection techniques and optimize their implantation process.

Materials and Methods

The stent manufacturing process consists of the following steps:

- Synthesis of the material,
- Modeling,
- Material processing,
- Laboratory tests,
- In vivo studies.

Results and Discussion

The obtained vascular stents are shown in FIGs. 1-4.

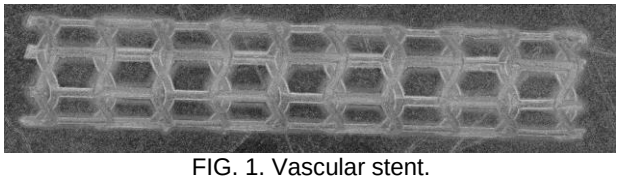

FIG. 1. Vascular stent.

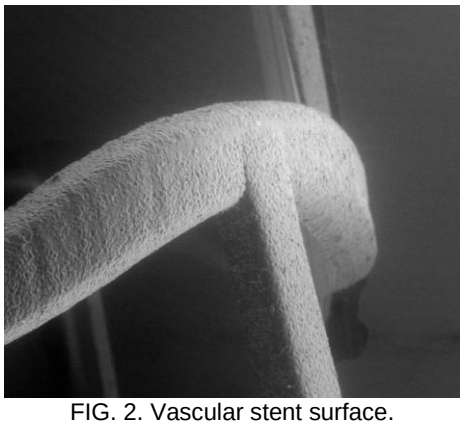

FIG. 2. Vascular stent surface.

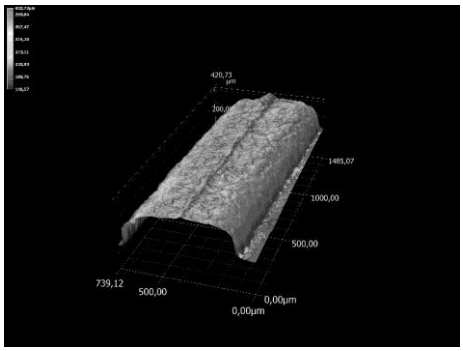

FIG. 3. Vascular stent surface.

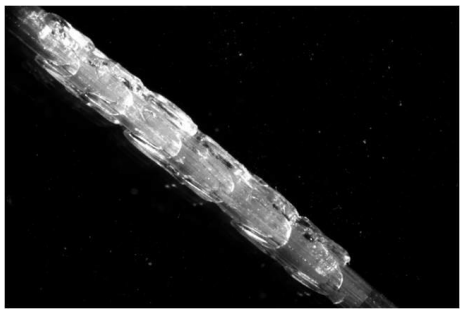

FIG. 4. Vascular stent clamped on the catheter.

Conclusions

The micro-injected stent clamped on the catheter and implanted according to the same procedure as for the implantation of commercially available stents, retains the shape memory effect that allows the stent to fit optimally to the vessel wall.

Acknowledgments

The research was carried out as part of project of The National Centre for Research and Development No. POIR.04.01.02-00-0105/17-00 "The technology for developing and obtaining the next generation of vascular stents through microinjection".
Thanks to Keyence for providing a VHX-7000 digital microscope for imaging of the received stents and their quality control.

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BIODEGRADABLE TRICALCIUM PHOSPHATE/POLY(3-HYDROXYBUTYRATE) SCAFFOLDS FOR BONE TISSUE REGENERATION

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[Engineering of Biomaterials 163 (2021) 74]

Introduction

Bioceramic scaffolds based on tricalcium phosphates (TCPs), despite their outstanding biological properties, exhibit low compressive strength and high brittleness. The fabrication of ceramic scaffolds in combination with degradable polymers may result not only in improved mechanical properties but also opens their potential application as carriers of biologically active substances supporting tissue regeneration [1]. Among different polymers used in tissue engineering, polyhydroxyalkanoates (PHAs) are of special interest as they are biocompatible, biodegradable and their degradation products (aliphatic (*R*)-3-hydroxy acids) are naturally present in cells that metabolize fats. To date, however, most research has focused on the use of PHAs in the soft tissue engineering [2]. In contrast, this research show the potential application of one of the PHAs, i.e. poly(3hydroxybutyrate) (P(3HB)) as a component of TCP/P(3HB) scaffolds for bone tissue regeneration.

The aim of this study was to obtain biodegradable TCP/P(3HB) composite scaffolds with different pore architecture. The influence of the polymeric coating on physicochemical properties of materials has been examined.

Materials and Methods

In our study, bioceramic scaffolds were prepared by a foam replication method. Three types of polyurethane matrices with different pore architectures were immersed in ceramic slurry (consisting of β-TCP powder, distilled water, Dispex A4040 and methylcellulose), dried and sintered at 1150°C. Then scaffolds were treated with 10% (w/v) aq. solution of citric acid for 3 minutes, thoroughly washed with distilled water and dried. Materials with small (TCP-S), medium (TCP-M) and large pores (TCP-L) were produced. The obtained ceramic specimens were infiltrated with 5% (w/v) P(3HB) chloroform solution, dried at room temperature for 7 days and subjected to further studies. The developed scaffolds were investigated by X-ray diffraction (XRD), scanning electron microscopy (SEM), hydrostatic weighing and compression tests. To evaluate the P(3HB) degradation, composites were incubated in distilled water at 37°C up to 180 days. Afterwards, extracts were analyzed *via* UHPLC-MS.

Results and Discussion

XRD analysis revealed that bioceramic scaffolds consist of one crystalline phase i.e. β-TCP. In the case of composites, β-TCP reflexes along with amorphous halo originated from P(3HB) were noticed. The obtained materials possessed open porosity around 65 vol% with spherical pores from 209 ± 87 to 714 ± 211 μm. SEM observations (FIG. 1) demonstrated that TCP scaffolds were uniformly covered with the polymer. Observed microporosity of P(3HB) layer is connected with fast chloroform evaporation and crystallization of the polymer. Composites possessed higher comprehensive strength (up to 4.5 ± 0.5 MPa) and surgical maneuverability in comparison to uncoated scaffolds (FIG. 2). Moreover, P(3HB) degradation products were identified by UHPLC-MS as (*R*)-3-hydroxybutyric acid and its oligomers, which can be beneficial for the surrounding tissues *in vivo* [3].

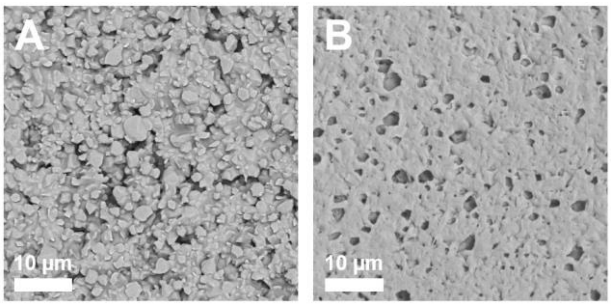

FIG. 1. Microstructure of materials: A) TCP-M and B) TCP-M/P(3HB).

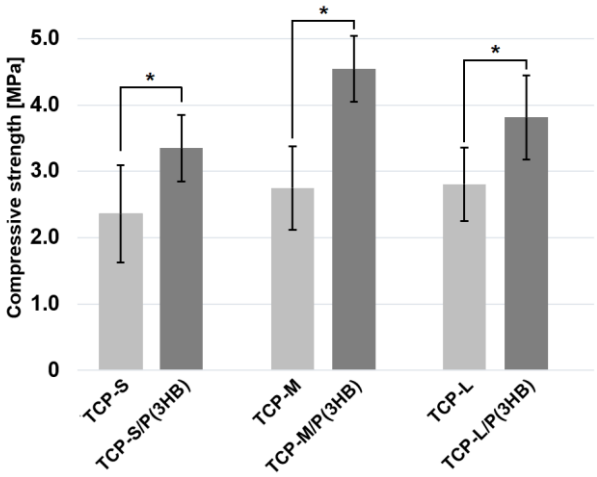

FIG. 2. Compressive strength of the scaffolds. Statistically significant differences are indicated by * $p \leq 0.01$.

Conclusions

The macroporous TCP and TCP/P(3HB) scaffolds with different pore architectures were successfully obtained. Polymer infiltration did not significantly affect open porosity of materials but improved their comprehensive strength. Moreover, degradation products of P(3HB) may be beneficial for the surrounding tissues as they can act as the nourishing agents. Thus, obtained composites were found to be promising bone substitutes for use in low-load bearing applications. Further *in vitro* studies are necessary.

Acknowledgments

Research funded by the National Centre for Research and Development, Poland, grant Techmatstrateg no. TECHMATSTRATEG2/407507/1/NCBR/2019.

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CARBON NANOMATERIALS BASED CHEMIRESISTIVE GAS SENSORS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 75]

Introduction

Due to their remarkable properties (electrical, thermal, mechanical, barrier) [1], carbon nanostructures become the subject of much contemporary research in material science, electronics, medicine and environmental protection. One of the enormous interests related to carbon nanostructures (especially graphene) is gas sensor development. The flexibility of using graphene and its derivates in various gas sensor architectures (chemiresistor, FET, electrochemical sensors) [2], large specific surface area, good electrical conductivity, easy synthesis, susceptibility to functionalization makes them a rising star of the gas sensor technology.

Nowadays, there is a lot of reports related to varieties of gases detectable by carbon nanostructure-based gas sensors. These materials can respond to low concertation of gases like VOC (volatile organic compounds), toxic gases [3] or traces of biological molecules [4]. One of the potential application in medicine is using carbon nanomaterial-based gas chemiresistor as a quick diagnostic tool similar to a breathalyser. The base concept is to detect low concentrations of metabolic products in human breath connected with human organism disorders like: presence of acetone related to diabetes (ketosis) [5] or the presence of ammonia accompanying chronic kidney disease [6]. Therefore, we would like to present the preliminary research about the possibility of using carbon nanostructured-based chemiresistor to detect ppm level concentrations of acetone.

Materials and Methods

Graphene oxide (GO) was prepared by the modified Hummers method [7]. GO was reduced by ascorbic acid (AA) on the ultrasonic bath at 60ºC for 60 minutes to partially reduced graphene oxide (pRGO). The effectiveness of the reduction process was evaluated based on XRD and EDS spectra. Next, carbon nanostructures prepared in this way were drop-coated onto interdigitated electrodes (Micrux ED-IDE1 Au).

Moreover, such interdigitated electrodes were overcoated by a thin (≈200 nm) diamond layer deposited in a microwave plasma-enhanced chemical vapor deposition system and terminated by hydrogen plasma.

Both gas sensors response measurement was carried out at room temperature and RH ~ 40%. The carrier gas was nitrogen, and the analyte was acetone. The measurement was performed for three different concentrations in a continuous experiment.

Results and Discussion

Spectroscopic techniques confirmed chemical changes that occurred in GO during oxidation and reduction processes. XRD diffraction spectra (FIG. 1) showed the presence of peaks typical for GO and pRGO.

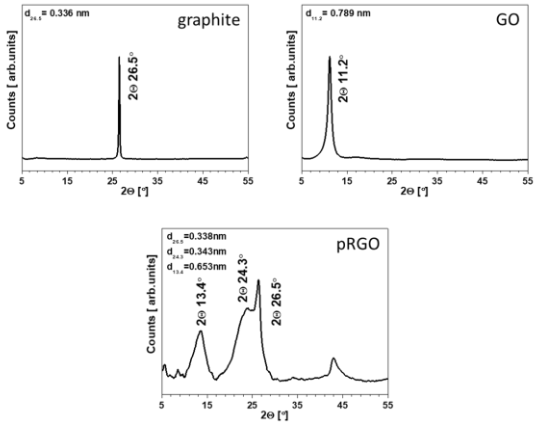

FIG. 1. Graphite, GO and pRGO XRD spectra.

EDX (TABLE 1) elemental analysis also confirmed partial reduction of GO with ascorbic acid. After the reduction process there was observed almost 50% reduction of oxygen atoms in carbon nanomaterial.

TABLE 1. EDX percent atomic composition of GO and pRGO.

Element	GO	pRGO
C [%]	77.40	90.72
O[%]	22.60	9.28

Preliminary gas sensor measurements showed promising results because the carbon nanostructure chemiresistors were able to detect acetone at ppm level in room conditions. The sensor response was stable during 5 h exposition of the material to the analyte, with slight sensitivity toward concentration changes.

Conclusions

To conclude, development of direct carbon nanomaterials functionalization towards response to specific gas/vapours is the tune of the future for gas sensors applications. This type of materials gives the promise to easily fabricated devices, working stable without the need of regeneration in opposite to commercial sensors, for instance metal oxides based. It reveals great potential in many fields like diagnostics or environmental monitoring, which are key factors in medical prophylaxis and fast diagnostics of human body dysfunctions.

Acknowledgments

P. S. Wrobel thank the National Science Centre of Poland for the financial support within the frame of the Preludium Program (grant agreement 2018/29/N/ST8/02356). O. Szabo and Š. Potocký acknowledge the GAV of the Czech Republic's Mobility project no. PAN-20-21. P. S. Wrobel, A. Kordyka and S. Pusz acknowledge the mobility project between the PAS and CAS (PAS-CAS project "Study of carbon-based sensors for detection of various gaseous media")

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THE CHARACTERIZATION OF SCAFFOLDS BASED ON DIALDEHYDE CHITOSAN/ HYALURONIC ACID

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[ENGINEERING OF BIOMATERIALS 163 (2021) 76]

Introduction

Hyaluronic acid (HA) is also known as hyaluronan, because in physiological conditions it exists in the form of a sodium salt, therefore negatively charged. Hyaluronic acid is a glycosaminoglycan copolymer of d-glucuronic acid and n-acetyl-d-glucosamine. HA has been shown to play an important role in lubrication, cell differentiation and cell growth. Its properties, both physical and biochemical, in solution or hydrogel form, are extremely attractive for various technologies concerned with body repair [1].

Periodate oxidation of chitosan has gained more attention in recent years. Periodate-oxidized chitosan has been described as a component for achieving biocompatible solid surfaces [2]. The process of periodate oxidation endows chitosan with multiple functional aldehyde groups. Hence, the aldehyde groups might react with the free amino groups within other substrates. The dialdehyde chitosan (DAC) is an effective cross-linking agent of collagen materials, cotton fabrics and chitosan/collagen/silk fibroin materials [2,3,4].

The aim of our study was to obtain scaffolds based on dialdehyde chitosan/hyaluronic acid mixture as a novel method of hyaluronic acid-based materials modification. Our study focuses on characterization of dialdehyde chitosan/hyaluronic acid scaffolds to be used in biomedical applications.

Materials and Methods

Reagents were purchased form Sigma-Aldrich (St. Louis, MO, USA). Dialdehyde chitosan was obtained by one step synthesis [2].

Dialdehyde chitosan and hyaluronic acid were dissolved separately in water at 1% concentration. Subsequently, substances were mixed in different ratios (w/w), and resultant solutions were homogenized on the magnetic stirrer for 1 h. Next, mixtures were poured into 24-well polystyrene culture plates, frozen, and lyophilized.

Obtained scaffolds were evaluated as described below.

Results and Discussion

Fourier transform infrared spectroscopy (FTIR) was used to observe chemical structure of scaffolds. Scanning Electron Microscopy (SEM) imaging was prepared to assessed microstructure of materials. 3D materials with highly porous structures are desired candidates for tissue regeneration where significant enhancement of the nutrient maintenance for targeted cutaneous cells is required. We also noticed that the resultant materials kept their shapes and homogeneity. The FTIR analysis allowed to observe the presence of functional groups in the DAC/HA scaffolds as well as shifts which may indicates the hydrogen interactions. Additionally human epidermal keratinocytes (NHEK) and dermal fibroblasts (NHDF) were used to evaluate of cell proliferation in presence of subjected scaffolds. It was found that scaffolds were characterized by porous structure with interconnected pores. There were no significant differences between cell proliferation in all scaffolds and this observation was visible in all subjected cell lines.

Conclusions

Scaffolds based on dialdehyde chitosan and hyaluronic acid were obtained. They had porous structure with interconnected pores. The material composition did not affect the cells viability. The porosity of material was around 90% what allow to classify it as highly porous and thereby suitable for the application in tissue engineering.

Acknowledgments

This research was funded by the Nicolaus Copernicus University in Torun, grant number: 282/2021 IDUB SD (B.K.S.) and to some part by the German Research Foundation (Deutsche Forschungsgemeinschaft [DFG]), grant number: KL2900/2-1 (K.K.) and by the National Science Centre, grant PRELUDIUM number 2018/31/N/ST8/01391 (S.G.Z).

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DETERMINATION OF HYDROCORTISONE RELEASE PROFILE FROM POLYMERIC NANOCARRIERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 77]

Introduction

In the preparation of controlled delivery system of active substance, the selection of an appropriate drug carrier is the most important. Currently, it can used the synthetic and natural biodegradable polymers, which occur in the nanometric scale. Moreover, polymeric materials which react to specific external factors, such as: temperature, pH, ionic strength, electric or magnetic field, light, and other chemical and biological stimuli, are particularly interesting. Due to they can be used in different area of medicine and pharmacy [1-4].

Materials and Methods

In this studies pH sensitive (poly(acrylic acid-co-methyl methacrylate) and temperature sensitive (N-isopropylacrylamide) polymeric nanocarriers were obtained by radical polymerization and the initiator of the reaction was ammonium persulfate (APS), while poly(ethylene glycol) diacrylate (PEGDA, Mn = 575 g/mol) or N, N'-methylenebisacrylamide were used as the crosslinking agent, respectively. After that, the encapsulation of the model active substance – hydrocortisone, was carried out. The hydrocortisone is a corticosteroid which helps to reduce swelling (inflammation) in the skin [5]. After, the encapsulation efficiency was assessed and the average particle size of the carrier – drug system was determined. In addition, various studies were carried out using the following research techniques: SEM, DLS and FT-IR, which allowed to analyze both the carrier and the carrier-drug system.
The release test was carried out using the Spectra/Por Standard regenerated cellulose (RC) membrane. Each dialysis bag was placed in a thermostatic chamber containing 250 ml of receptor solution. The assays were performed in buffer /ethanol solution (70:30 v/v) at pH 7.4, at 37°C for 7 days. The released concentration of steroid in the receptor solution was analyzed by means of UV-Vis spectroscopy (Perkin Elmer Company), at the wavelength of 245 nm.

Results and Discussion

The analysis of the obtained hydrocortisone release profiles from the systems containing the thermosensitive (FIG. 1) and pH-sensitive (FIG. 2) carrier showed a slow and prolonged release of the active substance - hydrocortisone. In the case of the pH-sensitive carrier - hydrocortisone system, the maximum drug release - 70%, occurred after 2,880 minutes (2 days). From then on, the second phase of drug release can be seen - slow, sustained release, maintained at an average of 65%. For the system with the same drug concentration but with a thermosensitive vehicle, the maximum release of hydrocortisone, ie 77%, was observed after 7,200 minutes (5 days). According to literature reports, the hydrocortisone-loaded nanomicelles containing dextran-PLGA copolymer (pH-sensitive system), also shows similar results, i.e. slow and sustained release of the drug [5].

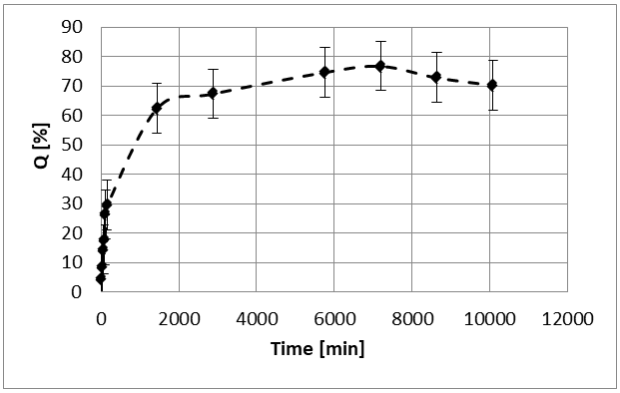

FIG. 1. The release profile of hydrocortisone from thermosensitive nanocarrier, at pH = 7.4 and T= 37°C.

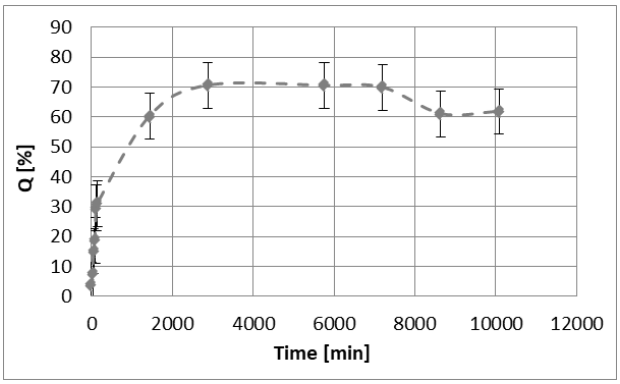

FIG. 2. The release profile of hydrocortisone from pH-sensitive nanocarrier, at pH = 7.4 and T= 37°C.

Conclusions

The results have shown that the pH-sensitive and thermosensitive polymeric nanocarriers developed in this study could be used as effective carriers for topical administration of hydrocortisone. Thanks to prolonged drug release profiles, it is possible to reduce the frequency of corticosteroid administration and the side effects.

Acknowledgments

This research was financial supported by The National Centre for Research and Development — project LIDER/41/0146/L-9/17/NCBR/2018.

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BIOMECHANICAL TESTING OF HYDROGEL DRESSINGS BASED ON SODIUM ALGINATE/ POLY(VINYL ALCOHOL)/ALOE VERA AND CONTAINING DRUG-LOADED INTO NANOCARRIERS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 78]

Introduction

The basic concept of application of wound dressing is based on the maintenance of moisture around the wound and the optimal absorption ability of the exudates from the wound surface which plays a critical role in the wound healing process. Accordingly, hydrogel materials are highly effective in the treatment of wounds. Sodium alginate (SA) presents high biocompatibility, hydrophilicity, and bacterial activity making it one of the most widely used polysaccharides polymers for hydrogel films preparation. However, alginate hydrogels may demonstrate undesirable mechanical properties, which significantly restricts their use as wound dressings. From this principle, the poly(vinyl alcohol) (PVA) has been utilized to improve the physical and clinical properties of blended polymeric materials. Recent developments in skin tissue therapy, tend to modify hydrogel materials with therapeutic agents capable of sustained and controlled compounds delivery to the wound site to promote the healing process [1-3].
In this research, a bio-hybrid hydrogel system, containing sodium alginate/polyvinyl alcohol and *Aloe vera* incorporating with the system of nanocarrier-active substances (salicylic acid, hydrocortisone, or fluocinolone acetonide), is presented as a potential skin dressing to accelerate wound healing.

Materials and Methods

In this work, the system of thermosensitive nanocarrier – drug was incorporated into SA/PVA hydrogel with *Aloe vera* content, were developed using chemical crosslinking method. Briefly, aqueous solution containing 2% (w/v) SA and 5% (w/v) PVA and 2% (w/v) of *Aloe vera* as well as poly(ethylene glycol) diacrylate (PEGDA, Mn=700 g/mol) and glycerine were thoroughly mixed. To prepare the hybrid system, pre-made drug-nanocarrier were introduced into the hydrogel precursor. After that, the prepared mixtures were heated and 4.4% (v/v) of ammonium persulfate were added. The properties of system of nanocarrier-drug-loaded hydrogel membranes such as the gel fraction, surface morphology and the static stretching test have been conducted.

Results and Discussion

The study showed that addition of salicylic acid (SA sample) noticeably decreased the gel fraction from 68% to 60%. It suggests that SA- drug system might reduce the entanglement reaction and consequently the gelation process is reduced slightly. The presence of hydrocortisone (H) or fluocinolone acetonide (F) does not significant changes in the hydrogels gelation process while the introduction of SA-F drug system lowers the value of the gel fraction to 62%.
The stretching tests shows the slight impact of added drugs on the mechanical properties of bio- hybrid hydrogel systems. All of the tested films are characterized by a medium elongation values of around 24–32% at break. As shown in FIG. 1 the lower elongation at break value was observed for hydrogel containing salicylic acid system while the addition of fluocinolone acetonide drug increased the tested parameter by approx. 6%, which makes it more flexible than a hydrogel without a drug.

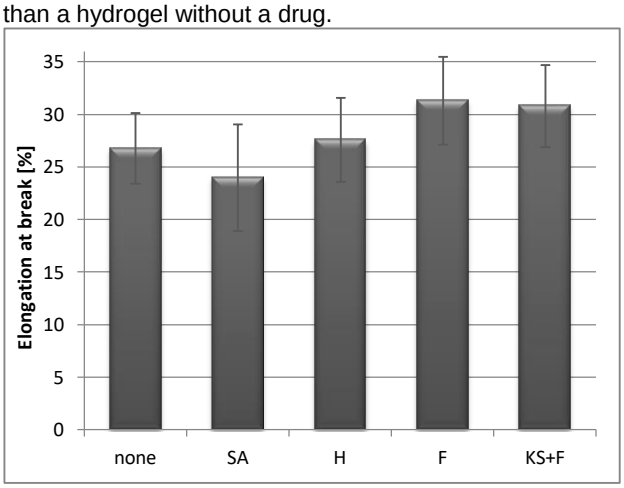

FIG. 1. Elongation at break in static stretching test of hydrogels.

Conclusions

1. SA/PVA/AV hydrogel films loaded with salicylic acid, hydrocortisone, or fluocinolone acetonide were obtained by chemical crosslinking method.
2. The SA/PVA/AV hydrogel membranes loaded with salicylic acid exhibited lower crosslinking density compared to films without drug system.
3. In the light of stretching tests, the introduction of nanocarrier-drug system into SA/PVA hydrogel with *Aloe vera* matrices did not reduce the mechanical parameter and thus the structure of the hydrogels is not disturbed.
4. Therefore, the system of nanocarrier-drug-loaded hydrogels based on sodium alginate/poly(vinyl alcohol) and *Aloe vera* are promising materials forwound dressing applications.

Acknowledgments

The research was financed by The National Centre for Research and Development in the frame of the 41/0146/L-9/17/NCBR/2018 project.

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INFLUENCE OF DUAL CROSS-LINKING ON THE SWELLING ABILITY OF GELATIN-ALGINATE HYDROGELS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 79]

Introduction

Hydrogels are three-dimensional materials capable of absorbing large amounts of water while maintaining their structure [1]. The swelling capacity is due to the high thermodynamic affinity of this class of materials to the solvent. Gelatin is a biopolymer, obtained by denaturation of collagen. As a polar biopolymer, gelatin dissolves quickly in water. However, when cross-linked, it gains better mechanical properties and reduction of degradation degree [2]. Alginates are naturally occurring anionic polymers commonly obtained from brown seaweed. The soluble alginate sodium salts are converted into insoluble gels by cross-linking with divalent ions [3]. Calcium chloride is one of the most widely used agents for the ionic cross-linking of alginate.

Materials and Methods

Gelatin and sodium alginate were dissolved in water and then mixed to finally obtain a solution of 6% gelatin and 2% sodium alginate in one mixture. Meanwhile, two different crosslinkers were prepared: squaric acid (SQ) in 2% and dialdehyde starch (DAS) in 1% and 2% weight percent based on the dry weight of the protein. Finally, the hydrogels were immersed in 1% or 2,5% and 5% calcium chloride solution to cross-link sodium alginate.
The swelling ability was tested using the conventional gravimetric method. Dried samples were weighed and placed in PBS solution at room temperature. The incubation process was held for 1, 2, 4, 6, 24, and 48 h.

Results and Discussion

The examined gelatin-alginate gels exposed high swelling ability. The swelling ratio observed after 24 h hydrogel incubation in PBS solution (FIG. 1) is presented below.

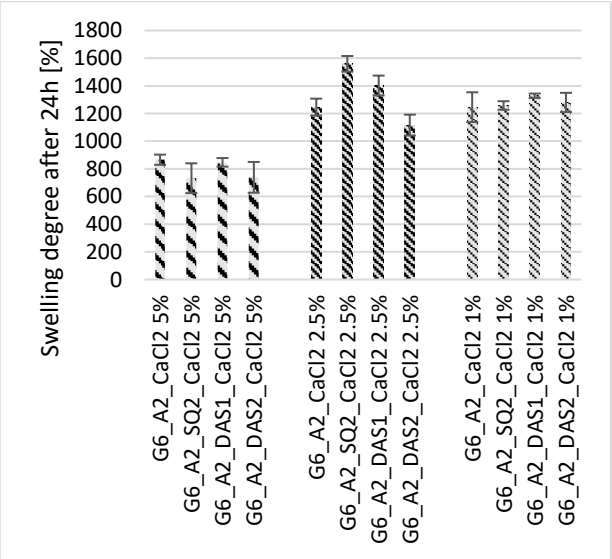

FIG. 1. The swelling ratio after 24 h hydrogel incubation in PBS solution.

Base materials G6_A2_$CaCl_2$ 1% and G6_A2_$CaCl_2$ 2.5% showed similar swelling ability after 24h incubation in PBS. However, the cross-linking by 5% $CaCl_2$ solution caused a reduction of swelling degree. After the 24 h incubation, the highest increase in the swelling ratio for G6_A2_SQ2_$CaCl_2$ 2.5% and G6_A2_DAS1_$CaCl_2$ 2.5%, was observed. The smallest increase in the swelling ratio was observed in all gels ionically cross-linked by 5% calcium chloride solution indicating an effective gelatin-alginate cross-linking process. These results show the greater effect of concentration $CaCl_2$ solution applied for cross-linking than the use of chemical cross-linkers DAS and SQ on the swelling degree. On the other hand, the hydrophilic nature of DAS and the polar structure of SQ could affect the increased water absorption capacity of the material.

Conclusions

The swelling ability test confirmed the integrity and stability of materials achieved by dual cross-linking of sodium alginate and gelatin. The swelling ratio significantly decreased after the addition of 5% of $CaCl_2$ solution. This result might be caused by the competitive interactions between Ca^{2+} ions and DAS and SQ, especially in higher concentrations. Also, the $CaCl_2$ solution concentration presented as more important than the addition of cross-linking agents.

Acknowledgments

The authors would like to thank the National Centre for Research and Development (NCBR, Poland, Grant no: TECHMATSTRATEG2/407770/2/NCBR/2020) for providing financial support to this project.

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PRODUCTION OF ENDOTOXINS BY *DESULFOVIBRIO DESULFURICANS* CELLS GROWING ON THE Ti6Al4V ALLOY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 80]

Introduction

The sulphate-reducing bacteria of the species *Desulfovibrio desulfuricans* colonize the human digestive system, being an element of the physiological microflora of the intestine [1-4]. However, like most intestinal bacteria, they become pathogens in specific conditions. The endotoxins secreted by these bacteria into the environment are among the threats [5]. Various strains of the *D. desulfuricans* species show the ability to colonize titanium alloys, creating biofilms on them [6,7]. Ti6Al4V alloy is one of the most widely used in implantology. Therefore, there is a direct risk to human health, resulting from the possibility of colonization of the titanium implant in the human body by the tested bacteria. The aim of this work was to check the endotoxins production capacity of three selected strains of *D. desulfuricans* bacteria grown on Ti6Al4V alloy surface.

Materials and Methods

The experiments have been conducted to investigate the endotoxins production capacity of three selected strains of *D. desulfuricans* bacteria: the standard DSM strain (isolated from soil) and two wild strains DV/A and DV/B (isolated from human intestine [4]). The bacteria have formed biofilms on an alloy of titanium with aluminium and vanadium (Ti6Al4V) under various conditions of the surrounding medium. In the experiments, the Ti6Al4V alloy samples with a ground surface were used. The research was carried out during the cultivation of bacteria in four different environments imitating human body fluids, which were: artificial saliva, artificial saliva simulating inflammation, as well as Tyrode and Ringer fluids. The samples were incubated for 28 days under the adopted conditions. Endotoxin (lipopolysaccharide; LPS) concentrations in bacterial cells were determined using Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit.

Results and Discussion

The obtained results of determination the LPS concentration in *D. desulfuricans* cells forming biofilm on samples of the tested titanium alloy are presented in FIG. 1 in the form of graphs, after subtracting the background value. The blank was sterile distilled water. The tests were performed in triplicate. The obtained results were averaged and recalculated, giving the results as the number of endotoxin units (eu) in a volume of 1 ml of biofilm suspension scraped from a single titanium alloy sample [eu/ml] ± average deviation. The ability of each of the three tested strains to grow, create a biofilm and produce endotoxins in all the tested culture conditions was found. Thus, the results confirm the natural presence of LPS in the cells of the *D.desulfuricans* bacteria. It was found that the differentiation of LPS production by three strains of *D. desulfuricans* bacteria creating biofilms on the alloy samples in solutions simulating body fluids was insignificant. Slight differences were only observed for the DV/B and DSM strains that formed biofilms in the presence of Tyrode's fluid. Besides, the DV/B strain showed the least stability in the production of endotoxins.

Conclusion

The results showed no statistically significant differences in the concentrations of endotoxins produced by bacterial strains that formed biofilms on the surface of the Ti6Al4V samples in the presence of the tested culture fluids.

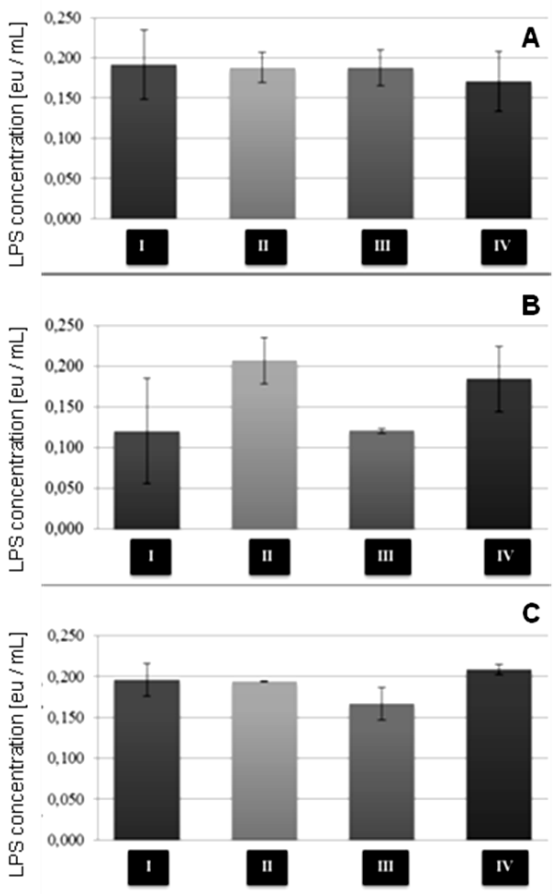

FIG. 1. Endotoxins (LPS) concentration in biofilms formed on the surface of the Ti6Al4V alloy samples by strains: A – DV/A, B – DV/B, C – DSM in various culture fluids: I – artificial saliva, II – artificial saliva simulating inflammation, III – Tyrode's fluid, IV - Ringer's solution.

Acknowledgments

This work was supported by the research project No N N518 291940 (Polish National Science Center). We thank D. Merkuda for participation in the analysis of endotoxins.

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BIORESORBABLE VASCULAR SCAFFOLDS - EFFECT OF COATING PARAMETERS ON MORPHOLOGY AND DOSE OF ANTIRESTENOTIC DRUG

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[ENGINEERING OF BIOMATERIALS 163 (2021) 81]

Introduction

Coronary artery disease (CAD) is the leading global cause of death, accounting for more than 9 million deaths per year according to World Health Organization estimates in 2016 [1]. Implantation of drug eluting stents (DES) has become the standard of care for patients undergoing percutaneous coronary intervention (PCI) [2]. Moreover, novel fully bioresorbable vascular scaffolds (BRS) have been designed to overcome the complications of metallic drug eluting stents (DES), e.g. vascular inflammation, hypersensitivity reactions and incidence of thrombosis [3]. However, the design of BRS continues to evolve with the intention to further improve short- and long-term outcomes. The scaffolds should restore luminal patency, but they can also act as a local delivery device for therapeutic agents. The use of biodegradable drug-eluting coatings are therefore being widely explored. The objective of this study was to develop degradable sirolimus-eluting polymer coatings applicable to bioresorbable polymer-based scaffolds by ultrasonic coating system and to identify parameters that may be used for tailoring the drug dose.

Materials and Methods

Cardiovascular scaffolds obtained by microinjection molding from poly(lactide-co-glycolide-co-trimethylene carbonate) (TMC) (Ø 5.4 mm) were used for coating study. The scaffolds were coated with sirolimus eluting layer composed of poly(L-lactide-co-trimethylene carbonate) (poly(L-lactide-co-TMC) (PLLA/TMC) by ultrasonic method using ExactaCoat (Sono-Tek) ExactaCoat (Sono-Tek) equipped with nozzle with Impact Ultrasonic Spray Shaping. Factors influencing the properties of coating layer were compared, e.g. concentration of polymer solution (1.0 % and 2.5 %), number of layers (3, 5 and 7) and type of nozzle (60 kHz and 120 kHz). The scaffolds were characterized for drug dose and morphology. Quantification of sirolimus in coating layer was performed at the wavelength of 287 nm using a high performance liquid chromatography (HPLC; LaChrom Elite®VWR/Hitachi, Tokyo, Japan). Morphology of scaffolds was observed by means of scanning electron microscopy (SEM; FEI Company, Quanta 250 FEG) and optical microscope (KEYENCE, VHX 7000).

Results and Discussion

The surface of bioreborbable cardiovascular scaffolds was modified by biodegradable polymer containing antirestenotic drug using ultrasonic coating system. The ultrasonic method enabled to form smooth coating, well-integrated with scaffold (FIGs. 1, 2). The amount of drug increased significantly in coating layer produced from 2.5 % polymer solution compared to 1.0% polymer. The coatings composed of 3 layers obtained from 1.0% polymer solution contained about 50 µg of sirolimus. Drug content increased to about 100 µg in 5 layers and to 140 µg in 7 layers. The coatings obtained from 2.5% polymer solution contained about 170 µg, 300 µg and 400 µg of sirolimus in 3, 5 and 7 layers, respectively.

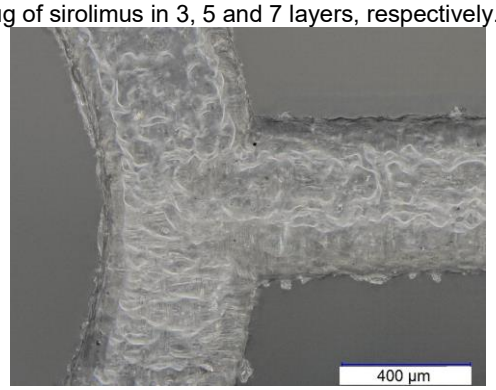

FIG. 1. Optical microscope image of the part of scaffold obtained from PLGA/oTMC coated with 7 layers of PLLA/TMC (1.0 %) and sirolimus using 60 kHz nozzle.

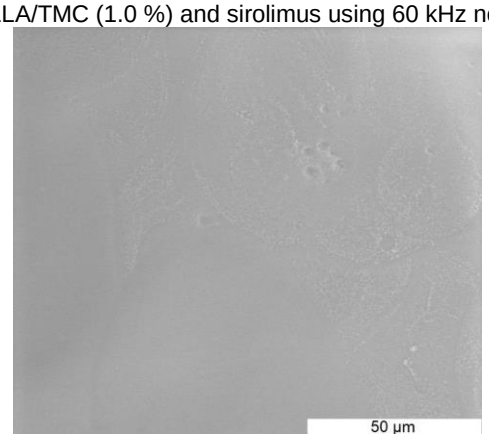

FIG. 2. SEM image of the part of scaffold obtained from PLGA/oTMC coated with 7 layers of PLLA/TMC (1.0 %) and sirolimus using 60 kHz nozzle.

Conclusions

Sirolimus-containing PLLA/TMC coating was developed for application by ultrasonic coating system. The drug content may be modified by number of layers and concentration of polymer solution.

Acknowledgments

This study was conducted in the frame of project of The National Centre for Research and Development No. POIR.04.01.02-00-0105/17 – MICROINJSTENT "The technology for developing and obtaining the next generation of vascular stents through microinjection".
The Authors thank to Keyence for providing a VHX-7000 digital microscope for imaging of the scaffolds.

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DEVELOPMENT OF POLYESTER COATINGS ON POLY(ETHYLENE TEREPHTALATE) PROSTHESES FOR VASCULAR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 82]

Introduction
Cardiovascular diseases are a serious problem nowadays, causing the highest percentage of deaths each year in the world. Despite successful application of commercially available vascular prostheses made of expanded polytetrafluoroethylene (ePTFE) or poly(ethylene terephthalate) (PET), there is a need of their surface modification, to improve cell adhesion and neointima creation [1]. Currently, innovative polyesters of citric acid and diols (polyalkylene citrates, PAC) are intensively investigated due to their biocompatibility with human tissues, controllable mechanical properties and degradation kinetics. The use of such polyesters could significantly improve functionality of vascular prostheses [2]. The aim of this study was to develop an optimal modification of vascular prostheses made of PET with poly(octamethylene citrate) (cPOC) in order to improve their biological properties.

Materials and Methods
Poly(octamethylene citrate) (POC) was synthesized in polycondensation reaction of citric acid and 1,8-octanediol in a molar ratio of 2:3, at 140°C for 40 min, and later it was purified and freeze-dried, to obtain POC prepolymer. Samples of PET prostheses (1 cm x 1 cm) were covered with an undiluted prepolymer (100%) as well as 30%, 15%, 7.5% and 2.5% ethanolic prepolymer solutions using dip-coating method and cross-linked at 80°C, 200 mbar for 10 days, resulting in crosslinked POC (cPOC) coatings on prostheses. The samples were weighed before and after the prepolymer application, as well as after cross-linking process to determine the final ratio of the cPOC coating. The effectiveness of polymeric coverage was tested using SEM and FTIR-ATR methods. For further studies involving L929 cells, the sample covered with 2.5% POC solution was chosen. Cells at a density $15x10^3$ were seeded on the samples unmodified and modified with cPOC, and also on the control sample (TCPS), and incubated at 37°C, 5% CO_2. After 1, 3 and 7 days the live-dead staining was performed and the cells were observed using fluorescence microscopy (Axiovert Zeiss 40 CFL). Metabolic activity test (Alamar Blue) was performed, and data were statistically analysed with t-test.

Results and Discussion
The weight of the cPOC layer on samples after crosslinking decreased significantly, what was caused by evaporation of the solvent and by elimination of water molecules during polycondensation of the prepolymer. Coating with more concentrated POC solution resulted in formation of thick layers comprising up to 40% of the total sample weight, what negatively affected the flexibility and shape of the prosthesis. 2.5% POC was found to be the optimal solution concentration for prosthesis coverage, resulting in a layer comprising of 10% of the total sample weight. FTIR-ATR studies confirmed the presence of cPOC on PET prosthesis. SEM pictures revealed that the coatings were inhomogeneous in some places. Biological studies with L929 cells showed no cytotoxic effect of the samples coated with cPOC polymer. The pH of the culture medium during incubation of the samples remained constant within the correct physiological range (~7). It suggests that potential release of POC unreacted monomers or degradation products did not cause acidification of the surrounding environment. Live-dead staining revealed a huge amount of living cells on the surface of the modified and unmodified protheses. The results of Alamar Blue test showed that cells grown on a modified sample exhibited slightly higher metabolic activity than those on the unmodified one. In addition, cells found on the bottom of the well were developing correctly, indicating that the presence of the samples did not have a negative and cytotoxic effect on cell proliferation.

Conclusions
In this work, an attempt to develop a stable cPOC coating on PET vascular prosthesis using dip-coating method was made. The best concentration of POC in ethanol to prepare coating was chosen and then used to produce the samples for biological studies with L929 cells. The polymer synthesis and the process of prosthesis modification resulted to be simple to carry out and the necessary reagents were cheap and easily available. SEM and FTIR-ATR studies confirmed presence of the coating on the PET prosthesis samples. Experiments with L929 cells showed no cytotoxic character of examined cPOC layer, however, there is a need to improve the process of seeding the cells on the material due to its hydrophobic character. Future research will be focused on cPOC modification aimed on the introduction of anti-oxidant properties.

Acknowledgments
This study was financed by the National Science Center, Poland (2018/28/C/ST5/00461), by the Program "Excellence Initiative – Research University" and from the subsidy of the Ministry of Education and Science for AGH-UST (No. 16.16.160.557).

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BIOLOGICAL PROPERTIES OF NANOSILVER-LOADED PMMA BONE CEMENT DOPED WITH BIOACTIVE GLASS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 83]

Introduction

Bone cements are an attractive biomaterial group dedicated for the treatment of bone fractures and fixing implants. One of their main representatives is acrylic cement based on poly(methyl methacrylate) (PMMA). PMMA cement is a bioinert material without osseointegrative as well as antibacterial properties, which may result in limited integration with host bone tissue, implant-associated infections, or even future ineffective biofunctionality [1]. Hence, to obtain a fully bioactive biomaterial, commercially available bone cement was modified simultaneously with antibacterial and osteocondutive agents. In this work, the influence of such modification on the biological properties of cement was evaluated.

Materials and Methods

Acrylic bone cement Cemex (Tecres, Italy) was used as the base material, modified simultaneously with nanosilver (AgNPs; 1.5 wt.%; particle size ~50 nm; MkNano, USA) and one of the following bioactive glasses (BG; 5 wt.%): 45S5 or 1393-B3 obtained by the traditional melting method (particle size ≤40 μm). All cement specimens with/without modifications were prepared as described earlier [1-5]. Briefly, the powders of modifiers were manually mixed with cement powder, and then the liquid cement component were added and a paste was obtained. The cement paste was then placed into the mold and allowed to cure. The following tests of cement's biological properties were performed: 1) hemolysis assay, 2) platelet aggregation, 3) periodontal ligament cells (PDLCs) viability and morphology, 4) bacterial growth inhibition (*S. aureus* ATCC, *S. aureus* hospital strain and *E. coli* ATCC) and 5) bactericidal effectiveness (*S. aureus* hospital strain). All specimens before the research were sterilized with 75% ethanol followed by 30 min exposure to UV light.

Results and Discussion

PMMA bone cement, despite its advantages, still has some essential drawbacks and as a bioinert material does not meet the requirements for modern biomaterials. The proposed modification of cement using nanosilver and bioactive glass may be a valuable alternative to the currently used in clinic cements (mainly doped with antibiotic/s). The addition of nanosilver should protect against infections, while the addition of bioactive glass was aimed at inreasing the release of AgNPs and also improving cellular response.

The obtained results showed that the incorporation of various bioactive glasses had different effects on the biological properties of modified bone cement. For all tested specimens, the hemolysis rate did not exceed 5% and LDH in the supernatant 15.0 μmol/min/10^{12} RBC, hence no severe hemolytic reaction was found. Exposure of platelets to the specimens did not induce their spontaneous aggregation and there were no significant changes in thrombin-induced aggregation, expect 1393-B3 glass addition, for which a significant reduction in platelets viability and aggregation was also found. The viability of PDLCs cultured on the tested specimens significantly decreased compared to that on neat cement. The addition of 1393-B3 glass decreased cell viability drastically (about ~95%), while the 45S5 glass slightly higher viability compared to nanosilver-loaded cement. The neat cement,, nanosilver-loaded cement and nanosilver-loaded cement with 45S5 glass did not affect the PDLCs morphology, and the confluent monolayer was observed, however for the nanosilver-loaded cement with 1393-B3 BG most of the cells were rounded and not adhering well to the material. The applied nanosilver to bone cement significantly slowed down the growth of bacteria, compared to neat cement as well as antibiotic. Further, the addition of BG significantly improved the cement antibacterial effectiveness due to the increasing release of AgNPs, especially in the case of 1393-B3 BG. Moreover, the bactericidal effectiveness against *S. aureus* hospital strain was confirmed as in bacterial culture after exposure to specimens the number of bacteria was significantly reduced and the almost 100% killing of bacteria was found for nanosilver-loaded cement with 1393-B3 BG.

Conclusions

Nanosilver-loaded bone cement doped with various bioactive glasses has been successfully fabricated and displayed different biological properties. The addition of AgNPs allows for active antibacterial protection, and BG improves its release. Depending on the type of bioactive glass, a different cellular response for nanosilver-loaded cement was found. Based on the results for potential medical applications, we recommend the AgNp-loaded BC with the 45S5 BG as bone substitute and AgNp-loaded cement with 1393-B3 BG as a coating for spacers or drug delivery system in therapy.

Acknowledgments

The authors would like to express their sincere gratitude to the Higmed Poland s.c. for providing bone cement for the research.

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FORMATION AND CHARACTERIZATION OF ANODIC TITANIUM OXIDE FILMS CONTAINING Ca, P AND Si IONS ON SELECTIVE-LASER MELTED Ti13Zr13Nb ALLOY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 84]

Introduction

One of the directions to improve the mechanical properties of titanium alloy dedicated for biomedical application is to use newly β-type titanium Ti13Zr13Nb alloys known as low modulus and bioinert metals. Together with selective laser melting (SLM), it's possible to obtain preferable mechanical properties of implants [1,2]. Further, to enhance the bone tissue response, surface modification is obligatory. According to the literature, the addition of Ca, P, and Si ions can significantly improve the bioactivity of titanium implants [3]. Among the surface modification method, micro-arc oxidation (MAO), has gained special attention due to capability to produce dense oxide film that binds well to substrate, enhances biocompatibility and bioactivity, coat the complex-shape objects and can incorporate ions into the structure.

The main aim of this work was to investigate the formation and characterization of anodic titanium oxide films containing Ca, P, and Si ions on selective laser melted Ti13Zr13Nb.

Materials and Methods

The titanium specimens were manufactured by the Realizer SLM 100. The MAO process was performed at two voltages of 300 and 400 V with a constant value of time and current equal to 15 minutes and 32 mA respectively, in an electrolytic solution containing 0.1 mol/L of calcium glycerophosphate (GP), 0.15 mol/L calcium acetate (CA) and two various contents of Na_2SiO_3.: 0.02 and 0.06 mol/L. The MAO process characterization was analyzed by the time-dependent (PCD-300A; Kyowa Electronic Instruments) relations of voltage and current. To obtain the characteristics of coatings, the microstructure (SEM JSM-7800F), topography, surface roughness, pore diameter (LSM; Olympus LEXT OLS4100 3D), thickness (Elcometer, 456, Elcometer Inc), elemental composition (EDS; S-3400NX, Hitachi), crystal structure (XRD, Bruker D8 discover), and surface wettability (optical tensiometer Attention Theta Life) were evaluated. The nanomechanical properties were determined using nanoindenter, while MAO coating adhesion properties were estimated by the scratch test (NanoTest Vantage, Micro Materials Ltd., Wrexham, UK). The ability of calcium phosphate formation on oxide coatings was examined to obtain the bioactivity characterization (Immersion test in Hank's solution).

Results and Discussion

Macro-porous, Ca- and P-containing titania-based films were successfully formed on the Ti13Zr13Nb alloy substrates. The phase, Ca, P and Si content, morphology, roughness, thickness, nanomechanical properties, and adhesion of the MAO coatings were strongly dependent on the applied voltage. Due to the good ratio of structural and nanomechanical properties of the coatings, the optimal conditions of the MAO process were found at 300 V, which resulted in the predictable structure, high Ca/P ratio, the highest demonstrated early-stage bioactivity, better nanomechanical properties, elastic modulus, and hardness close to the values characteristic for bones. The addition of 0.02 M Na_2SiO_3 improves critical load of adhesion and total delimitation, while with increasing content of silica, the contact angle and the Ca/P ratio in the structure also increased.

Conclusions

The combination of SLM and MAO strategy is the perspective method to improve nanomechanical and bioactive properties of titanium alloys for biomedical application.

Acknowledgments

This research was supported by Tokyo Medical and Dental University and funding by NAWA program.

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MECHANICAL PROPERTIES OF WIRES FOR MEDICAL USE MADE FROM MODIFIED 316LVM STAINLESS STEEL

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[ENGINEERING OF BIOMATERIALS 163 (2021) 85]

Introduction

Stainless steel is a material commonly used for medical implants used in orthopaedics and traumatology [1]. This steel is used for the production of unblocked nails for bone osteosynthesis [2]. Current solutions used in bone osteosynthesis can be either external or internal. In the case of the internal technique, implant-assisted anastomosis blocks the possibility of bone elongation, which is especially important for patients in the growing phase [3]. Hence, the production of 316LVM steel wires with modified properties will make it possible to introduce them into the production of bone nails/wires to treat patients in the growth phase. The work aimed to produce charging wires for the production of implants made of 316 LVM steel and characterize their mechanical properties.

Materials and Methods

The elemental chemical composition of stainless steel 316LVM. for intramedullary nails/wires, was prepared based on the requirements of ISO 5832-1. The ingots were made in the Valbruna steel plant using an electric arc furnace (EAF). Then they were remelted in a vacuum arc furnace (VAR) followed by hot forged. Hot forging was applied to produce a rod with a diameter of 5,5 mm. Their surface was etched with a mixture of sulfuric acid, hydrogen peroxide, and Cleanox and Brightner prepare. Then, in the BHH Mikrohuta Sp. z o.o., they were drawn into the form of wires with a final diameter of 1,2 mm.
The mechanical properties of rod/wires were determined based on the results of static tensile tests performed using the Inspekt table 50-250 kN machine (Hegewald und Peschke MPT) following the standard PN-EN ISO 6892.

Results and Discussion

Characteristics of the stages during the wire production were made based on measurements of its diameter. In FIG. 1, the relationship between wire diameter and total reduction (Z) as well as the total deformation (ε_s) were summarized. In the applied technological process, the total maximum reduction of 95% was obtained with a wire diameter of 1,2 mm. This reduction in wire diameter resulted in a total deformation of 3.
However, the most important aspect of plastic working was to obtain information on the mechanical properties of the wires. Based on the results from the static tensile test, the yield point ($R_{0,2}$) and the ultimate tensile strength (R_m) were determined for each wire (FIG. 2).
It can be seen that as the total reduction of the wire diameter increases, both the yield point and the ultimate tensile strength increase. It is a consequence of the change in grain morphology - their fragmentation and elongation in the direction of wire drawing as well as an increase in the density of dislocations generated in cold working.

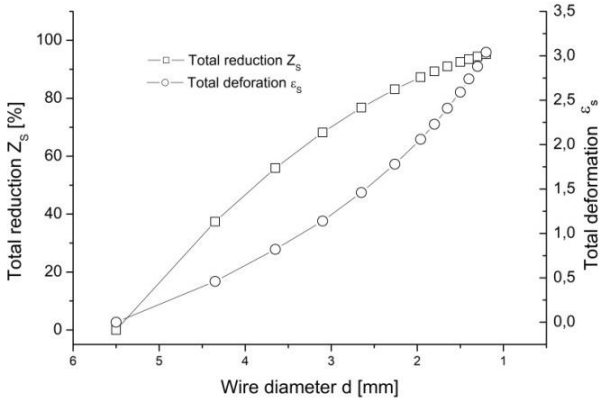

FIG. 1. Relationship between wire diameter d and total reduction (Z) and deformation (ε_s).

From the point of view of the applicability (of the wire for intramedullary nails and wires), the requirements of the standard, regarding ultimate tensile strength, must be met. In FIG. 2, the requirements for wires and nails were marked depending on the diameter of the wire. These conditions are met for wires with a diameter of 2,0 - 1,8 mm and 1,5 mm. In the case of nails, these conditions are met by all produced wires with a diameter less than 3 mm.

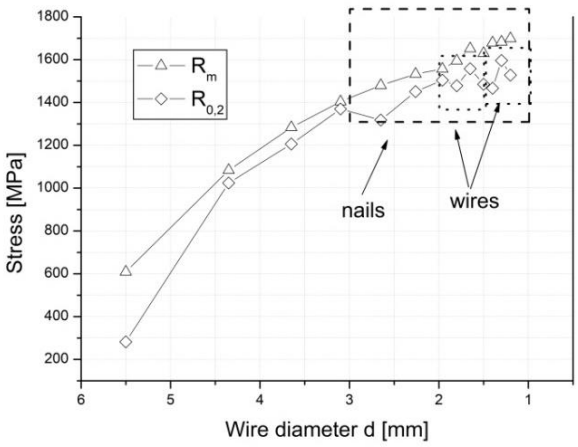

FIG. 2. Relationship between total reduction (Z) and yield point ($R_{0,2}$) and the ultimate tensile strength (R_m).

The mechanical properties of the wire with a diameter of 2.6 mm are noteworthy. The difference between the tensile strength and the ultimate tensile strength is 162 MPa. Such conditions increase the possibilities of cold plastic processing.

Conclusions

The applied methods of producing modified stainless steel 316 LVM and the ingot processing enable the production of the starting material for the manufacturing of rods and wires that meet the requirements of standards for medical devices. In addition, this material can be successfully used for the production of intramedullary wires and pins.

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INHALABLE DRUG DELIVERY SYSTEM BASED ON POLY(SEBACIC ACID) AND AZITHROMYCIN FOR THE TREATMENT OF BACTERIAL INFECTIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 86]

Introduction

Bacteria-related respiratory infections are commonly occurring in patients suffering from e.g. chronic obstructive pulmonary disease or cystic fibrosis [1]. As a conventional treatment, i.e. oral or intravenous administration of antibiotics, which are burdened with a number of negative side effects followed by limited bioavailability of the drugs, novel methods of treatments allowing for more effective delivery of the antimicrobials to the site of action are being developed [2].

The aim of this study was to develop an inhalable drug delivery system based on poly(sebacic acid) (PSA) microparticles loaded with azithromycin (AZ). The MPs should be spherical with diameters in 1-5 μm range, as such particles are the most effective for deposition in lower respiratory tract when delivered via inhalation [3].

Materials and Methods

PSA microparticles (MPs) were fabricated using oil-in-water emulsification/solvent evaporation method. In brief, 60 mg of PSA was dissolved in 3 ml of dichloromethane (DCM) and mixed with 3 mg (5% w/w), 6 mg (10% w/w) or 12 mg (20% w/w) of AZ. As prepared oil phase was then emulsified into 20 ml of aqueous 8% w/v poly(vinyl alcohol) solution on magnetic stirrer (1500 rpm). After 5 h, the suspension of MPs was collected, centrifuged and rinsed with deionized water (3x). Purified MPs were frozen at -80°C and freeze-dried for 24 h.

The MPs were characterized in terms of their morphology (scanning electron microscopy – SEM), size, polydispersity and zeta potential (dynamic light scattering – DLS) and AZ encapsulation efficacy (high-performance liquid chromatography – HPLC). Degradation of selected MPs (i.e. unloaded MPs and those containing 10% w/w AZ) was also evaluated for up to 96 h. Finally, cytocompatibility of the MPs was determined in contact with human lung epithelial cells of malignant (A549) and non-malignant (BEAS-2B) origin via resazurin reduction assay and live/dead fluorescent microscopy staining.

Results and Discussion

SEM investigation revealed that all types of unloaded MPs and those containing 5-20% w/w AZ were spherical in shape with a smooth appearance at micrometre scale (FIG. 1). The average diameters of MPs ranged from 1.5 ± 0.1 μm (MP+10%AZ) to 2.7 ± 0.2 μm (MP+5%AZ), as evidenced by DLS. No particles smaller than 0.9 μm or larger than 4.1 μm were found, thus the MPs should be suitable for inhalation. Zeta potential of the freeze-dried MPs was negative for all samples (-15.5 to -6.3 mV). Due to hydrophobic nature of both PSA and AZ, the encapsulation efficacy was close to 100% as measured by HPLC.

The degradation behaviour of unloaded MPs and MP+10%AZ was evaluated. The pH of phosphate buffered saline (PBS) decreased significantly even within the first 2 h of degradation of both types of MPs in comparison to PBS alone (around 7.3 for unloaded MPs and 6.9 for MP+10%AZ). In the course of time, further drop in pH was observed for both samples to below 5.4 for unloaded MPs and 5.7 for MP+10%AZ after 96 h of degradation. The morphology of the MPs also changed during the degradation. It was observed that with longer incubation time, the MPs become smaller, less regular and their surface began to wrinkle. The changes were more pronounced in the case of MP+10%AZ, than in unloaded MPs.

Cytotoxicity tests were performed first for unloaded MPs at concentrations ranging from 0.1 μg/ml to 1000 μg/ml. It was found out that BEAS-2B cells were more sensitive to MPs than A549. Statistically significant differences in viability were forum at ≥500 μg/ml for A549 and ≥100 μg/ml for BEAS-2B. The second evaluation focused at comparison of cytotoxicity of unloaded MPs and AZ-loaded MPs at one concentration. None of the MPs showed cytotoxicity against A549 cells, while in the case of BEAS-2B the decrease in viability was observed in MP+20%AZ.

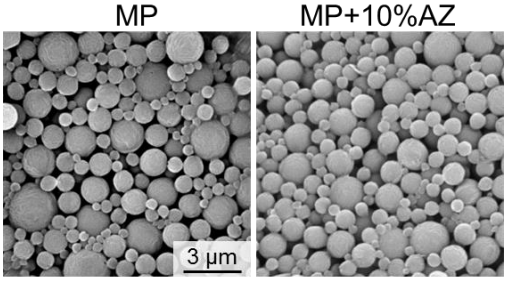

FIG. 1. SEM images of unloaded MPs (left) and MPs containing 10% AZ (right).

Conclusions

Oil-in-water emulsification/solvent evaporation method was suitable for fabrication of PSA-based microparticles loaded with azithromycin. The morphology and size of all types of MPs were suitable for administration via inhalation, as the MPs were spherical in shape, smooth and their diameters were in 1-5 μm range. The encapsulation efficacy of the drug was close to 100%. The MPs underwent rapid degradation as evidenced by changes in their morphology and decrease of buffer pH due to the release of acidic degradation products. The MPs were cytocompatible with human lung epithelial cells of malignant and non-malignant origin. Further studies on azithromycin release kinetics and antimicrobial efficacy of the MPs are necessary to fully evaluate the potential of the developed drug delivery system in the treatment of bacterial infections in lungs.

Acknowledgments

This study was supported by National Science Centre, Poland (project No 2019/35/B/ST5/01103) and by the Program ”Excellence Initiative – Research University” for the AGH University of Science and Technology.

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MECHANICAL PROPERTIES OF PHYSICALLY MODIFIED BACTERIAL NANO CELLULOSE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 87]

Introduction

Bacterial nanocellulose (BNC) is a material which has application in medicine. BNC has very good biological properties like non-toxicity, biocompatibility, bio-functionality, hypoallergenicity and well adsorbed so most often it is used for wound dressing materials [1]. BNC after synthesis process has high water content which is associated with low tensile strength. The higher tensile strength of BNC would allow it to be used as a material for artificial heart valves and blood vessels. Physical or chemical modification can improve the mechanical properties of the BNC. The aim of the work was to create a physical modification of BNC and check its mechanical properties.

Materials and Methods

Bacterial nanocellulose produced by BoWil Biotech Company according to the procedure described in the PL 171952 B1 was used. Modification consisted of convective drying in 25°C and then re-soaking in distilled water for 2 hours. After modification tensile strength test according to ASTM modified method D882-00, using 5543 Universal Testing Machine (Instron C., Canton, MA, USA) was carried. Initial grip separation was 50 ± 5 mm, and cross-head speed was 10 mm/min. BNC samples were strips whose dimensions were 15 by 100 mm. Measurements of the tensile strength (TS) and elongation at break (A) for all of the other samples were carried out immediately after the removing of the samples from water.

The nanoindentation test was carried out using a nanointender (Micro Materials, United Kingdom). Loading and unloading nanoindentation test using a Berkovich indenter was performed. The maximum load was constant and it was 5 mN and test applied load rate 0.01 mN/sec. The study provided information about the hardness of the material (H) and its reduced Young's modulus (E).

Tear test was carried out according to PN-EN ISO 9073-4:2002 for 10 samples using an INSTRON model 1112 testing machine.

A cavitation test was carried out in Ringer solution. The distance between the grips was 75 mm and the feed rate was 100 mm/min. The tip of the sonificator vibrated at 24 kHz. Cavitation tests were performed at the maximum vibration amplitude. The BNC sample was placed 0.5 mm from the vibrating tip of the sonificator.

Results and Discussion

Tensile strength of native BNC was about 2 MPa and after modification it increased to 18.3 MPa, which similar corresponds to Ul-Islam *et al.* [2]. This could be caused by a change in the degree of crystallinity index, the appearance of compressive stresses and a change in the spatial arrangement of the polysaccharide chains in the material. This has also been confirmed by physicochemical tests. Obtained TS values are also several times higher than the tensile strength of pig tissues of the circulatory system [3]. The hardness of BNC determined during the nanoindentation test is 0,26 MPa and reduced Young's Modulus about 4 GPa.

Below we can see the table with the obtained results of mechanical properties.

	Test	
	Tensile strength	**Nanoindentation**
Physical modyfied BNC	TS= 18.3 MPa	H= 0.26±0,01MPa
	A= 9.5 %	E= 4.13±0,31 GPa
	Tear	**Cavitation**
	R_{rd} =858 N/mm	Weight loss=0,04%

After physical modification, BNC has a higher tensile strength than natural pig tissues, so this material was additionally subjected to a tear test and cavitation resistance test. The tear strength (Rrd) was calculated in newtons per mm of thickness of the torn sample in accordance with of the formula: Rrd = P/g, where P is the maximum force which the sample was torn and g is the arithmetic mean of the sample thickness from three measurements. The average tear strength of the modified BNC was 858 N/mm. The standard deviation was 184.5 N/mm. The tear test result of the physically modified BNC was compared with the tear strength of the human aorta reported by Carson and Roach [3]. According to them, the tear strength of the human aorta is 15.9 ± 0.9 J/cm^2. Since 1 J/cm^2 corresponds to 10 N/mm, the tear strength of the human aorta is 159 N/mm. Comparing the tear strength of the human aorta and the physically modified BNC, it can be concluded that BNC has over five times greater tear resistance than the tissue which the human aorta is made from.

As it is considered that BNC could be a material for coatings of artificial heart valves, a cavitation resistance test of this material was also performed. Cavitation was carried out for 1.5 h. During this time, the BNC sample was taken out of the cavitation station every 30 minutes, dried in compressed air and weighed. The first weight loss of the sample, caused by cavitation loads, was recorded after 90 minutes of the cavitation test. After 90 min of the cavitation test, the measured weight loss was 0.04%, what is good result for overload conditions.

Conclusions

BNC after physical modification consisting in convection drying at 25°C and re-soaking in water, has good mechanical properties which could meeting the requirements for biomaterial for applications in cardiac and vascular surgery.

Acknowledgments

This work was supported by the Polish national research budget, under the National Centre Research and Development grant number PBS2/A7/16/2013.

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COMPUTER-AIDED ANALYSIS OF THE INFLUENCE OF PSEUDOINTIMA FORMATION ON TEXTURED IMPLANT SURFACES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 88]

Introduction

Modifications of the biomaterials surface in medical applications currently offer enormous possibilities for the specialization and functionalization of biomaterial-tissue connections. Appropriate selection of physicochemical properties and topography of the surface allows for a stable connection of the implant with the peri-implant tissue. In the clinic the use of a porous layer of hydroxyapatite in cementless endoprostheses increases the biomechanical potential of contact at the implant-bone interface and affects the rate of protein adsorption. In Mechanical Circulatory Support (MCS) devices textured surfaces has proven that appropriate topography allows controlled scar tissue formation, which results in a reduction of inflammatory processes and limitation of thromboembolic events. The interaction of an implantable biomaterial with living tissue is a very sensitive phenomenon, which in a very short time triggers a cascade of events resulting in cell attachment. The high energy at the implant/tissue interface is stabilized by the adsorption of ions, molecules and/or macromolecules such as proteins. The development of a natural protein biofilm on the surface of the implant allows for increased biocompatibility. The use of porous surfaces in MSC devices in the dynamic blood flow channels allows pseudointima formation, which results in a reduction of shear stresses. Pseudointima is resistant to thrombogenesis and in currently used constructions its formation is promoted by titanium microspheres and a fibrillar texture. The developed neointimal surface is mainly composed of collagen as well as cells derived from circulating progenitors of fibroblasts, myofibroblasts, monocytes, macrophages and endothelial cells. The biological mechanism of pseudointima formation is a complex phenomenon. There are also no reports in the literature describing this phenomenon using computer-aided numerical methods to enable optimization of surface parameters at the device design stage.

Materials and Methods

As part of the project, numerical analyses were performed to assess the impact of pseudointima formation on shear stresses in a simplified blood flow system. The main goal was the development of discrete phase model to simulate the phenomenon of backfilling porous surface by red blood cells (RBC) during blood flow. The blood behaviour in Langrangian view was examined on the basis of the particle tracking of RBC of blood plasma flow. However, the blood plasma behaviour was considered in Eulerian view based on the assumption of a finite volume (FV) element in the fluid flow path. During all analysis, many models of lab-on-chip were developed including three FV models to compare the effect of boundary conditions with and without roughness and their influence on blood velocity and generated shear stress. Moreover, four discrete phase (DP) models were created to compare the roughness effect and irregular morphology of surface on RBCs concentration during blood flow, velocity and time of particles distribution. Simulations were performed in Ansys software. The mesh of FV model of lab-on-chip (μ-Slide I Luer channel, IBIDI) [1] was composed of about 1 milion nodes. The mesh quality parameters - assessed on the basis of skewness, aspect ratio and element quality - were excellent. There were six layers of prism elements near walls of the model. The pressure-based solver and absolute velocity formulation were selected for computational purpose. The pressure-velocity coupling scheme of the solution was selected for computation with spatial discretization using gradient and transient formulation. Discrete phase model [2] was applied in Ansys Fluent and RBCs are able to interact with blood plasma [3]. The diameter of RBCs is assumed from 5 μm to 10 μm using statistical diameter distribution and the average diameter is 7.5 μm [4].

Results and Discussion

The introduction of roughness as a boundary condition in the DP model was preceded by an analysis of its influence on the computed results using FV method model of blood flow applying non-Newtonian power law. The influence of roughness on RBCs flow results using DP models was more visible than in the analysis of blood flow results using FV method models. The values of velocities and shear stress were significantly decreased in DP models of lab-on-chip with roughness. On the other hand, the concentration of RBCs was increased in the case of DP model with roughness near the bottom plane of the middle channel. The comparison of results reached by applying DP lab-on-chip model with cubes and without them indicates the significant decrease in values of shear stress and velocity, as well as the significant increase in values of particle concentration.

Conclusions

The introduction of roughness as a boundary condition in the FV method lab-on-chip model led to a reduction in shear stresses and a slower flow. The introduction of the same roughness parameters as a boundary condition in the DP method lab-on-chip model also led to a reduction in shear stresses and flow limitation, which additionally resulted in an increase of DP model concentration of the RBCs in the bottom part of the model. The arrangement of cubes on the lower surface of the lab-on-chip channel with a height of more than 100 μm organizes the flow of RBCs. The cubes have a strong influence on shear stress, velocity and DP model concentration of particles. The values of shear stress and velocity are decreased, whereas the values of particle concentration increased.

Acknowledgments

The work was sponsored from the project "Patient-specific, anti-microbial bioactive finger implants for durable functional reconstruction after amputation" (Project Acronym (Short name): fingerIMPLANT, by the National Center of Science and Development no. M-ERA.NET2/2019/7/2020

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MODERN DRESSING AND COSMETIC MATERIALS BASED ON HYALURONIC ACID MODIFIED WITH EGG ALBUMIN

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[ENGINEERING OF BIOMATERIALS 163 (2021) 89]

Introduction

For many years, hyaluronic acid has been one of the most frequently used natural polymers in cosmetics and biomedical materials due to its many valuable properties [1]. Hyaluronic acid can be modified with natural and synthetic polymers and substances of natural origin [2]. Modifications of hyaluronic acid can allow the creation of new and environmentally friendly biomedical or cosmetic materials that can be widely used in pharmacy, cosmetology, and medicine. Egg albumin obtained from an egg can accelerate the process of wound healing [3]. Therefore, a mixture of hyaluronic acid and albumin can be used as biomedical dressings.
The research aimed to check the interaction of hyaluronic acid with egg albumin and the mechanical properties of mixtures and pure substances.

Materials and Methods

Hyaluronic acid (HA) with three different molecular weights was purchased from Zrób Sobie Krem, Prochowice, Poland. Egg albumin was purchased from Sigma-Aldrich Sp. z o.o., Poznań, Poland. A 1.5% high molecular weight HA solution, a 1.5% low molecular weight HA solution, and a 1.5% solution in ultra-low molecular weight HA in water were prepared. 25g of each hyaluronic acid solution was poured onto plastic plates and the polymer film was allowed to dry for a week. The polymer films were then removed from the plates and subjected to FTIR analysis using a ThermoFisher Scientific Nicollet IS10 spectrometer. Fittings were cut from each film and then rip up a ZwickRoell testing machine to test the mechanical properties. Mixtures were prepared by mixing 25 g of a hyaluronic acid solution with 0.25 g of albumin and poured onto plates. Fittings were cut from the formed films, rip up at the machine, and subjected to FTIR analysis.

Results and Discussion

FIG. 1 shows the IR spectra of 1.5% high molecular weight HA and a mixture with 0.25 g ovalbumin. The black color shows the spectrum of 1.5% high molecular HA, and the grey color of the mixture. The obtained IR spectra show the differences between pure HA and its modification with albumin. We observe the shift of the bands in the spectrum and the increase in absorbance at the wavenumber 2929 cm^{-1}.
FIG. 2 shows the IR spectra of 1.5% ultra-low molecular weight HA and a mixture with 0.25 g of albumin. The black color shows the spectrum of 1.5% ultra-low molecular weight HA, and the grey color of the mixture. On the obtained spectra, one can see the shift of the spectrum of the mixture relative to the spectrum of the pure HA, which shows the interaction between the components of the mixture. One can observe a decrease in absorbance at the wavenumber 609 cm^{-1} and at the wavenumber 1039 cm^{-1}.

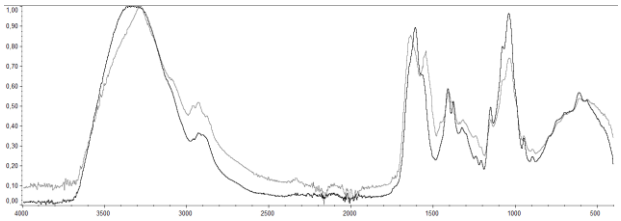

FIG. 1. IR spectra for 1.5% high molecular HA and 1.5% high HA with 0.25 g of albumin.

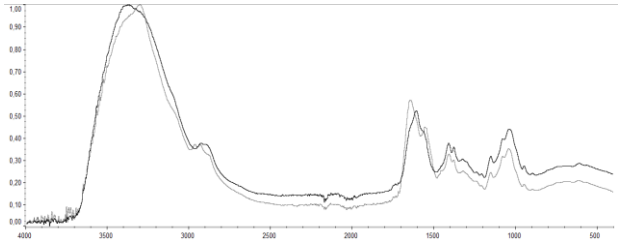

FIG. 2. IR spectra for 1.5% ultra-low molecular weight HA and 1.5% ultra-low molecular weight HA with 0.25 g of albumin.

Conclusions

Modification of hyaluronic acid with egg albumin leads to the creation of a new biomaterial significantly different from pure substances. FTIR analysis confirms the interactions between hyaluronic acid and albumin and the differences between mixtures of hyaluronic acids with three different molecular weights.

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MODIFICATION OF CHITOSAN FILMS WITH FISH SKIN COLLAGEN

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[ENGINEERING OF BIOMATERIALS 163 (2021) 90]

Introduction

Chitosan is a biopolymer obtained from chitin by its alkaline deacetylation. Due to its unique properties, it is practically used in many areas such as medicine, pharmacy, the cosmetics and food industry, and many others. Chitosan is used in various forms, such as solution, film, foam, fibers, scaffolds, etc. [1].

Collagen is a protein that is also widely used in medicine, cosmetics and food, as well as chitosan. Usually, collagen is obtained from the skins of land animals such as cows or pigs, but due to the possibility of transferring bovine spongiform encephalopathy (BSE) and transmissible spongiform encephalopathy (TSE), alternatives are sought. Hence the interest in fish collagen increases, however, the disadvantage of such collagen is the low denaturation temperature [2].

Mixtures of collagen and chitosan are not found in nature but can be obtained, among others, due to the presence of hydrogen bonds [3].

The aim of this study was to investigate the effect of fish collagen addition on the properties of chitosan films.

Materials and Methods

Collagen was purchased by WellU Sp. z.o.o, Gdynia, Poland. It was obtained by isolation collagen from the skin of *Silver Carp*. The skins were removed manually and washed with chilled tap water to get rid of the adhering tissues. The next stage was disinfection of the material using 3% hydrogen peroxide in water. Then leached its residue. Purified skins were placed in lactic acid solution for 3 days to extract the collagenous proteins. Then obtained solution was pressed through a material which allows collagen separation. The samples were then placed in polyethylene bags and stored at –25°C until used.

Medium and low molecular weight chitosan was purchased from Sigma-Aldrich.

2% solutions of both chitosans and a 20 mg/mL collagen solution were prepared using 0.5M acetic acid as solvent.

Mixtures of low and medium molecular weight chitosan with collagen were prepared in the following ratios: 25:75; 50:50; 75:25.

Solutions of both pure chitosans, pure collagen, and each mixture were poured onto plastic plates to form films.

The resulting films were analyzed with a PIKE GladiATR NICOLET iS10 FTIR spectrometer to obtain IR spectra.

The mechanical properties of each film were tested with a Zwick & Roell testing machine.

Results and Discussion

FIG. 1 shows the IR spectra of the three films. The first is made of a 20 mg/ml collagen solution, the second is made of a 2% medium molecular weight chitosan solution, and the third is a 50:50 mixture of the aforementioned chitosan and collagen.

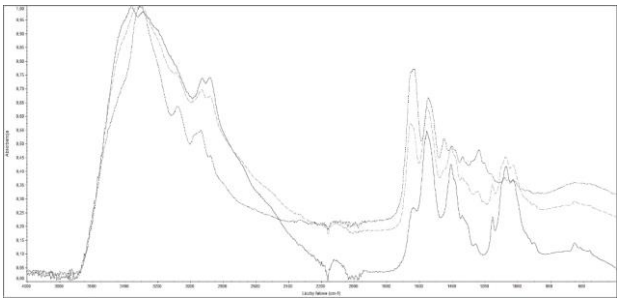

FIG. 1. IR spectra: collagen (dotted line), medium molecular weight chitosan (solid line), 50:50 mixture of chitosan and collagen (dashed line).

From IR spectra one can see that the interactions between two biopolymers, namely collagen and chitosan are due to hydrogen bonds. The shifts of amide bands in IR spectra have been observed.

Low molecular weight chitosan differs in mechanical properties from medium molecular weight chitosan.

The addition of collagen influences the mechanical properties of chitosan in various ways, depending on its molecular weight and share in a given mixture.

Conclusions

IR spectroscopy showed interactions between chitosan and fish collagen. Modification of the mechanical properties of chitosan films by fish collagen depends on the molecular weight of chitosan.

Acknowledgments

Authors acknowledge WellU company for the preparation of collagen for this study.

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THE PROPERTIES OF NANOSILVER AND NANOCOPPER – DOPED NANOHYDROXYAPATITE COATING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 91]

Introduction

Titanium and its alloys are nowadays the most frequently used materials for orthopedic implants because of their appropriate mechanical properties, high corrosion resistance, and biocompatibility [1].

To improve proper cells response and ensure adequate mechanical properties, surface modification techniques are applied including deposition of calcium phosphate coatings, usually hydroxyapatite coatings. Electrophoretic deposition technique is in particular suitable to develop thin films on implants. To provide antibacterial properties of the hydroxyapatite coatings, the doping of nanosilver or nanocopper nanoparticles with similar bactericidal effects as antibiotics was investigated [2,3].

In the present research, the effects of nanoAg, and nanoCu together on the nanoHAp coating properties, especially the mechanical properties, were investigated and discussed.

Materials and Methods

The specimens of Ti13Zr13Nb alloy were polished with abrasive paper, grid 2000# as the last.

The electrophoretic deposition (EPD) was carried out in a suspension prepared by dispersing 0.1 g of HAp nanopowder with an average particle size of about 20 nm (MK Nano Canada) (specimen A), 0.005 g of nanosilver with an average particle size of about 30 nm, and 0.005 g of nanocopper with average particle size about 80 nm (Hongwu International Group LTD, China) in 100 mL of anhydrous ethanol. The suspensions were obtained by ultrasonic mixing for 60 min at room temperature. The electrodes were placed parallel to each other within a distance of 10 mm and connected to the DC power source (MCP/SPN110-01C, Shanghai MCP Corp., China). The electrophoretic deposition was performed at 30 V for 2 min at room temperature. Specimen were air-dried at room temperature for 24 h. Finally, the coated Ti13Zr13Nb specimens were thermally treated in a tubular furnace (PROTHERM PC442, Ankara, Turkey) in a vacuum at 800°C for 120 min.

The nanomechanical studies were conducted with the nanoindenter (NanoTest Vantage; Micro Materials, UK) equipment using a Berkovich indenter. The maximum force of 5 mN, the loading, and unloading times 20 s each, the dwell period at full load 10 s, and the distance between the subsequent indents 20 μm were set up in nanoindentation tests. The Oliver-Pharr method was used to calculate the contact area of the indenter. The nanoscratch tests were performed at the maximum load of 400 mN and loading rate of 1.3 mN/s at a distance of 1000 μm. In the nanoscratch test the spherical diamond indenter with a 5 μm radius was used. The adhesion of the coating was determined as corresponding to the abrupt change in friction force.

Results and Discussion

For nanoHAp and nanoHAp/nanoAg/nanoCu coatings, the obtained values of nanohardness were 0.032 ± 0.009 GPa and 0.067 ± 0.055 GPa, respectively. Large standard deviations are characteristic for nanoindentation measurements of porous materials like the tested nanoHAp-based coatings. Furthermore, the addition of nanometals in our tests resulted in a change of hardness from 0.03 to 0.07 GPa, Young's modulus from 5 to 12 GPa, and H^3/E^2 from 1.25 to 1.01 MPa, which could be assumed as a substantial effect. FIG. 1. shows the maps of the distribution of Young's modulus for nanoHAp and nanoHAp/nanoAg coatings. The mechanical properties of implant coatings are expected to be close to those of human cortical bone [4].

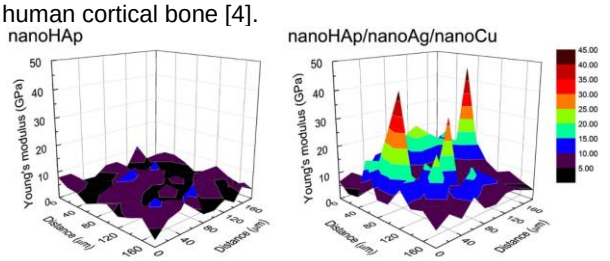

FIG. 1. The maps the distribution of Young's modulus for nanoHAp and nanoHAp/nanoAg/nanoCu coatings.

The nanoscratch-test studies proved the positive effect of the addition of nanomemetals. The value of the critical force of 106.77 ± 37.51 mN and 220.91 ± 62.19 mN, respectively for the nanoHAp and nanoHAp/nanoAg/nanoCu coatings were obtained. The single nanoscratch test curve for nanoHAp/nanoAg/nanoCu coating is presented on FIG. 2.

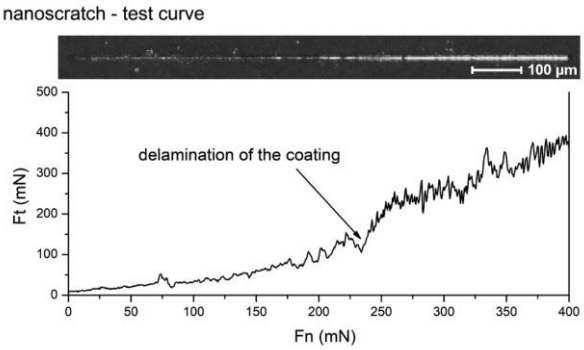

FIG. 2. Nanoscratch test curve.

Conclusions

The addition of nanometals positively influenced the nanomechanical properties, including the adhesion of the coatings determined by the nanoscratch test .

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CHARACTERISTICS OF PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF CHITOSAN HYDROGELS BASED ON SELECTED HYDROXYACIDS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 92]

Introduction

Chitosan is a natural cationic polymer that dissolves in an acidic environment and forms gels. Its properties depend on the degree of deacetylation and molecular weight. It is a bioactive compound which increases the regenerative abilities of the skin by stimulating the division of fibroblasts. Chitosan has antibacterial and film-forming properties. Moreover, it is biodegradable, biocompatible, non-toxic and stable. In the research mandelic and lactobionic acids were used. They are characterized by biological activity and low toxicity. This combination not only has a positive effect on the solubility of the polymer but also allows to obtain new biomaterials in which the positive features of the base ingredients are enhanced by their synergistic effect. The obtained hydrogels were assessed for the interaction of chitosan and hydroxyacid molecules, and the stability of the resulting structures was examined. The research was performed by using rheological methods and IR spectroscopy. [1-4]

Materials and Methods

Chitosan powder (low molecular weight, degree of deacetylation DD = 78%, M_v = 1.4×10^6 g/mol) was obtained from Aldrich and used without further purification [1]. Hydrogels were prepared by dissolving chitosan (2.6% w/v) in 30 mL of aqueous solutions of mandelic acid and lactobionic acid. The content of hydroxyacids was 0.002 mol [1]. The samples were mixed on a magnetic stirrer until clear solutions were obtained. After 24 hours of incubation, viscosity measurements were made at the temperature of 25°C in the range of the shear rate from $0.1s^{-1}$ to $35s^{-1}$. The rotational viscometer SMART series (Fungilab) and a set of appropriate spindles were used for measurements.
The structure of chitosan, mandelic and lactobionic acid as well as the interaction between them were confirmed by infrared spectroscopy using Nicolet iS10.

Results and Discussion

As a result of rheological studies, the dependence of dynamic viscosity on the shear rate (viscosity curves) was obtained, which allowed to conclude that hydrogels based on chitosan and mandelic acid are characterized by higher viscosity values compared to those containing lactobionic acid.
After a week of observations of the prepared hydrogels and measurements of viscosity parameters, it was noticed that the viscosities of the hydrogels were constantly increasing (FIGs. 1 and 2).

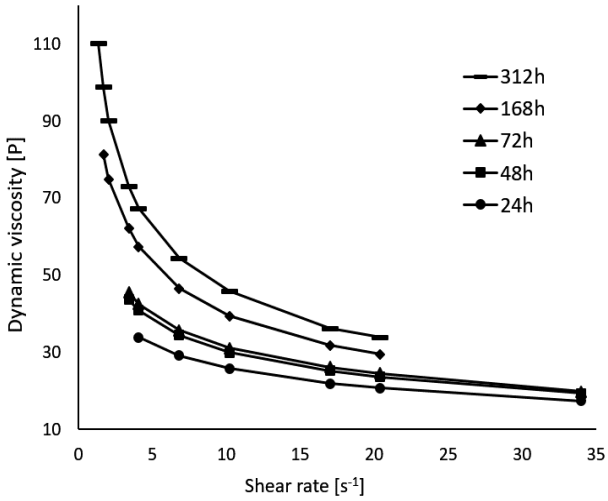

FIG. 1. Comparison of dynamic viscosity of chitosan gel with mandelic acid depending on the time

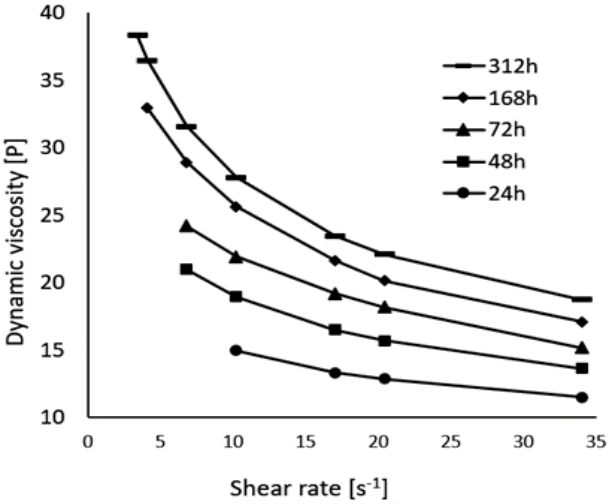

FIG. 2. Comparison of dynamic viscosity of chitosan gel with lactobionic acid depending on the time

It proves the ongoing process of creating new bonds between hydroxyacids molecules and chitosan chains. After this time, the hydrogels with mandelic acid showed higher viscosity values compared to hydrogels made with lactobionic acid.
Based on the obtained IR spectra, the shifts of the characteristic chitosan bands as a result of interaction with the tested hydroxyacids were analyzed.

Conclusions

Chitosan hydrogels made with the use of mandelic acid are characterized by higher viscosity values compared to hydrogels containing lactobionic acid. The samples of the obtained hydrogels stored for 7 days show no signs of degradation and their viscosities are constantly increasing.

Acknowledgments

Financial support from a student mini-grant obtained from Collegium Medicum, Nicolaus Copernicus University is gratefully acknowledged.

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CHARACTERISTICS OF SWELLING AND MECHANICAL PROPERTIES OF CHITOSAN FILMS BASED ON SELECTED HYDROXYACIDS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 93]

Introduction
Chitosan is a polymer with versatile properties. It is used in many areas, including medicine, pharmacy and cosmetology. In combination with hydroxyacids that enable it to dissolve, it creates spatial structures with potential applications in tissue engineering, dressing materials and drug delivery systems. Important features of hydrogels made from the combination of chitosan and hydroxyacids, which are important for the mentioned applications, are their swelling capacity (this means the ability to absorb large amounts of water without disturbing the three-dimensional structure), sorption properties towards various substances, for example, those that are components of exudate and their mechanical properties [1-3].

Materials and Methods
LMW chitosan (low molecular weight, degree of deacetylation DD = 78%, Mv = 1.4×10^6 g/mol) was used to obtain chitosan films used for further research. Chitosan (2.6% w/v) was dissolved in 30 ml of aqueous solutions of lactobionic and mandelic acids, 0.002 mol each. 1% glycerin was added. After incubation for 24 hours, the resultant hydrogels were poured 25 g into square Petri dishes (10 cm x 10 cm) and allowed to dry. The finished films were carefully removed from the plates and cut into pieces having an average weight of about 25 mg. The film fragments were placed in a 3% albumin solution, water and an aqueous lysozyme solution (1 mg/7.5 ml) for 1 to 5 hours. Each piece was weighed before being immersed in the solution and then pulled out and dried. The degree of film swelling was calculated.
The mechanical properties of the obtained films were tested with a Zwick and Roell testing machine (Ulm, Germany) [4].

Results and Discussion
The tested films showed a different degree of swelling depending on the solution in which they were immersed.
The films with lactobionic acid showed higher swelling parameters in the albumin solution. In contrast, in the case of water and lysozyme solutions, the films with mandelic acid swelled more. In each test solution, films containing mandelic acid were degraded faster (FIG. 1).
As a result of the mechanical tests, Young's modulus and the maximum tensile force at break were measured.

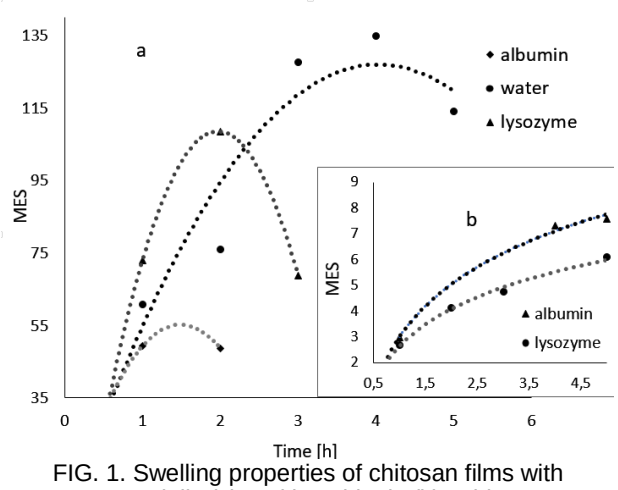

FIG. 1. Swelling properties of chitosan films with mandelic (a) and lactobionic (b) acids.

Conclusions
Films with lactobionic acid swelled less than with mandelic acid, at the same time showed better mechanical properties and were more resistant to dissolution and degradation processes.

Acknowledgments
Financial support from a student mini-grant obtained from Collegium Medicum, Nicolaus Copernicus University is gratefully acknowledged.

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SPECTROSCOPY ANALYSES OF STRUCTURE-PROPERTY CORRELATIONS IN CITRATE-BASED ELASTOMERIC BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 94]

Introduction

Biomaterials engineering remains one of the most rapidly expanding fields of science combining the achievements of medicine, biochemistry, materials science and polymer chemistry [1]. Particularly, in cardiovascular tissue engineering there is a need to design biomaterials with better performance and biological activity while maintaining appropriate mechanical and chemical properties. In this field, poly(alkylene citrates) (PAC) are a promising alternative for currently used biomaterials with their superior bio- and hemocompatibility, tuneable mechanical properties and ease of functionalization [2-3]. However, challenging structural characterization of PAC prepolymers has been reported in only a few reports despite the extensive published literature, with the majority of papers showing serious incoherencies in the discussion of the reported spectroscopic results [4-5]. Therefore, in this study, we present a novel comprehensive approach towards the spectral characterization of PAC biomaterials which led to structural impact on the materials properties exposure.

Materials and Methods

PAC prepolymers were synthesized in polycondensation reaction of citric acid (CA) and 1,8-octanediol (poly(octamethylene citrate) (POC)) or 1,6-hexanediol (poly(hexamethylene citrate) (PHC)) in a molar ratio of 2:3 or 1:1, at 140°C for 40 min, and later they were purified and freeze-dried. Obtained prepolymers were subjected to detailed NMR analyses using JNM-ECZR500 RS1 500 MHz spectrometer (Jeol, Japan). ^{1}H and ^{13}C, ^{1}H-^{1}H COSY, ^{13}C-^{1}H HSQC and HMBC experiments were conducted for prepolymers obtained in 1:1 initial molar ratio; for the 2:3 prepolymers only ^{1}H experiments were conducted. 60 mg of each prepolymer was used for analyses. On the basis of the obtained results monomers molar ratio estimation, degree of conversion (DOC) and citrate carboxylic group reactivity calculation were performed.

Results and Discussion

In accordance with the combined results of the 1D and 2D NMR analyses all of the prepolymer structures were elucidated and thoroughly described thus eliminating the incoherencies present in the existing reports. Calculations performed on the ^{1}H NMR spectra revealed the reagents molar ratio in the prepolymer structure as similar to the molar ratio of the comonomers during the synthesis, which led to the conclusion of the importance of the strict control of the polycondensation condition. It was observed, that ^{13}C NMR spectra of POC_1:1 show differences in signals originating from substituted and unsubstituted citrate carboxylic groups. After the analysis of ^{1}H and HMBC spectra it was found that in the studied samples terminal carboxylic groups of CA show higher reactivity than the central group (~20% for POC and ~40% for PHC). It strongly indicates that the formation of linear oligomers over branched ones during the initial stages of polycondensation of CA and specific diol is favoured. The oligomers linearity directly implicates the cross-linking mechanism and provides the unique mechanical properties of the PAC materials, as well as has an impact on their biocompatibility. Calculated conversion degrees indicate a high extent of carboxyl consumption (up to 84% for PHC_1:1) which is independent of the length of diol but is strongly affected by the molar ratio of reagents used during prepolymer synthesis (~82% for POC_1:1 vs. ~65% POC_2:3).

Conclusions

In this work, we presented a novel approach to the NMR spectroscopy application in the PAC biomaterials characterization. Such profound NMR analyses for PAC materials have not been reported to date. All of the prepolymer structures were elucidated and re-evaluated in the contrary to the existing literature reports. The main conclusion is the exposition of a number of possibilities associated with the characterization of PAC properties via the detailed investigations of NMR results. The understanding of the reactivity of citrate carboxylic groups indicated the priority of the formation of linear oligomers, which directly translates into cross-linking density as well as mechanical and biological properties of the materials obtained. The confirmation of the molar ratios of the reagents demonstrated the importance of redesigning the prepolymer synthesis protocol as well as explained differences in prepolymers solubility and led to the estimation of the appropriate polycondensation time.

Acknowledgments

This study was financed by National Science Centre, Poland (2018/28/C/ST5/00461), by the Program ”Excellence Initiative – Research University” and from the subsidy of Ministry of Education and Science for AGH-UST (No. 16.16.160.557). Further support was provided by the Czech Science Foundation (grant No. 21-06065S). Martina Trávníčková received funding (Development of HR capabilities, internationalization, popularization and IP utilization, No. CZ.02.2.69/0.0/0.0/16_028/0006226) for traveling to Poland.

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THE ENCAPSULATION OF ANTIBACTERIAL DRUGS IN POLYMER NANOPARTICLES AND THEIR USE IN DRUG DELIVERY SYSTEMS ON ZrO_2 SCAFFOLD WITH BIOACTIVE COATING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 95]

Introduction

Bone infections are relatively common problem, and at the same time their treatment is difficult, due to the limited ability of antibiotics to accumulate in the bone [1]. There is also an increase in infections of joints, particularly after their surgical treatment. This is due to an aging population that results in a reduced immune response, more hip fractures, and more frequent use of invasive joint surgery, with the risk of microbial infections. Bacteria can cause serious damage to bone tissue as well as spread to neighbouring tissues and organs [2,3].
The aim of this study was to: 1) bioactivate the ZrO_2 bioinert scaffolds using the biomimetic calcium phosphate (CaP) layer deposition method, 2) create polymer nanoparticles (NPs) of poly(L-lactide-co-glycolide) loaded with antibacterial drugs and 3) immobilize NPs on ceramic scaffolds, using the drop-casting method and incorporating NPs during the deposition process of the bioactive layer.

Materials and Methods

The nanoparticles were obtained using the double emulsion method with solvent evaporation. Gentamicin (gent) and bacitracin (Bct) were used as antibacterial drugs. NPs shape and size were characterized using scanning electron microscopy (SEM) and dynamic light scattering (DLS), respectively. Ceramic scaffolds were made by pressing and sintering method and then immersed in ten-time concentrated solution of simulated body fluid (10xSBF). The quality and thickness of the deposited layers were tested by means of SEM. The effectiveness of the immobilization of NPs by both methods and their adhesion to the substrate after incubation in a simulated biological environment was also checked.

Results and Discussion

The results showed that the proposed method is effective in NPs preparation. NPs are of spherical shape and their size is ca. 200 nm. The high efficiency of encapsulation and changes in surface potentials have been confirmed by the OPA and Zeta potential tests, respectively (TABLE 1). The result of NPs immobilization was confirmed by SEM (FIG. 1).

TABLE 1. NPs characterization.

	PLGA	PLGA_gent	PLGA_Bct
Average size [nm]	226,3	207,4	202,4
Zeta potential [mV]	-30,5	-23,9	-13,4
Encapsulation [%]	--	54,3	37,5

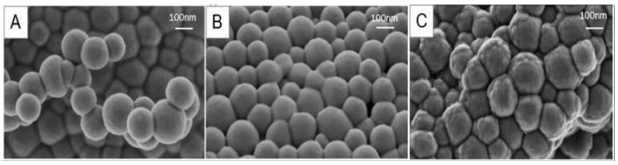

FIG. 1. NPs SEM characterization A. PLGA, B. PLGA-Gent, C. PLGA-Bct.

The microstructure of the deposited layers was examined by SEM. The bioactive layer consisted of flakes-like mineral crystals (FIG. 2).

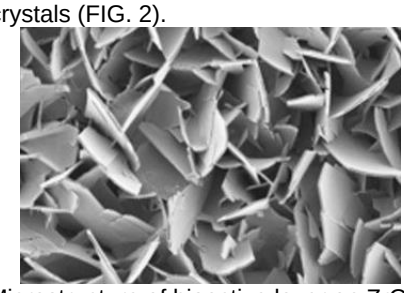

FIG. 2. Microstructure of bioactive layer on ZrO_2 scaffold.

NPs were deposited on the scaffolds by two methods: co-precipitation in SBF and by drop-casting. As SEM microphotographs show (FIG. 3) both methods seem to be suitable do deposit NPs on CaP bioactive layer.

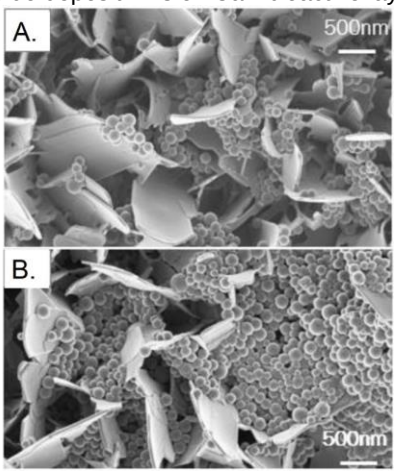

FIG. 3. SEM microphotographs of NPs deposited on ZrO_2 scaffolds during the CaP layer preparation process (A) and by drop-sitting method (B).

Conclusions

The presented processes of surface bioactivation and immobilization of NPs are effective in creating bioactive ceramic scaffolds that can be used in tissue engineering and as drug delivery systems to treat bone infections.

Acknowledgments

This study was financed by National Science Center, Poland (2016/21/D/ST8/01685)

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POLYHYDROXYALKANOATES - PRODUCTION STRATEGIES AND THEIR APPLICATION IN MEDICINE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 96]

Introduction

Polyhydroxyalkanoates (PHA) are natural polyesters with unique properties, valuable for the environment, medicine, pharmacy and industry. They are a diverse group of biopolymers characterized by biodegradability and biocompatibility, and additionally, they are often made of industrial, petrochemical or municipal waste [1]. PHAs are most often produced by fermentation in the cells
of many strains of environmental bacteria. They are composed of (*R*)-3-hydroxy acids, which makes them an additional rich source of optically active compounds [2]. PHAs can be composed of over 150 different monomers, which makes them a reservoir of biopolymers with various properties. PHAs with up to five carbon atoms in their monomer molecule belong to the group of short-chain polyhydroxyalkanoates (scl-PHA). They have been used mainly in industry as biodegradable polymers for food packaging [3,4], but also as hard materials in tissue engineering, soluble sutures or bone implants. Medium chain length polyhydroxyalkanoates, mcl-PHAs (C6 – C14 carbon atoms) are biocompatible elastomers, therefore they are good materials for biomedical applications for soft tissues. They are also widely used as drug carriers [3,4]. Soft, hydrophilic aromatic polymers such as poly(3-hydroxy-5-phenylvalerate) (PHPV) have been used successfully to regenerate cortical neuronal cells [5]. In the presented research, two types of mcl-PHA polymers of medical importance are produced: aliphatic poly(3-hydroxynonanoate) (PHN) and aromatic PHPV by using two different biosynthesis strategies.

Materials and Methods

Two bacterial strains were used for the biosynthesis of polymers: *Pseudomonas putida* CA-3 (synthesis of aromatic PHA) and *Pseudomonas putida* KT2440 (synthesis of aliphatic PHA). PHPV synthesis was performed by pulse-fed flask cultures (400 ml of cultures in 2L flasks) in MSM medium supplemented with trace elements (TE), where the carbon source was the sodium salt of 5-phenylvaleric acid (40 mM). The flasks were incubated for 48 h at 30°C with shaking (250 rpm). Then, another portions of sodium salt of 5-phenylvaleric acid (40 mM) and trace elements were added to the culture and incubated under the same conditions for another 48h (limited amount of nitrogen in the medium step) [5]. The aliphatic polymer PHN was produced under fully controlled conditions in a 5L reactor (Biostat® B, Sartorius) using the *P.putida* KT2440 strain. The culture medium was also MSM with the addition of TE and $MgSO_4$ (0.2 gL^{-1}). The carbon source for the bacteria was a mixture of nonanoic acid and butyric acid (4: 1), fed automatically throughout the duration of the culture. The pH of the culture was kept constant at 6.9. Phosphate deficiency was the limiting factor in the medium. The cultivation was carried out for 30 h at 30°C with stirring and aeration. Both polymers were extracted with ethyl acetate from the resulting bacterial dry matter. Then the solvent was evaporated and the polymers were precipitated in cold methanol and dried. Biosynthesis efficiency, PHA content in bacterial dry matter and polymer purity were assessed by performing analyses using HPLC-MS/ MS (Agilent 1290 Infinity System with MS Agilent 6460 Triple Quad Detector) and GC (Varian CP-3800 with FID detector).

Results and Discussion

Two PHA polymers were obtained: aromatic PHPV and aliphatic PHN using two feeding strategies. The obtained results are shown in TABLE 1.

TABLE 1. Comparison of the pulse-fed flask reactor and fed-batch reactor feeding strategies for two bacterial strains (*P. putida* CA3 and *P. putida* KT2440) in the production of PHPV and PHN.

Strain	*P.putida* CA3	*P.putida* KT2440
Carbon source	sodium salt of 5-phenylvaleric acid	nonanoic acid/ butyric acid
Reactor type	pulse-fed flask reactor	fed-batch reactor
Limiting factor in MSM medium	nitrogen deficiency	phosphate deficiency
PHA content [%]	57%	71%
Polymer type	homopolymer	heteropolymer
Polymer composition [%]	**100%** of 3-hydroxy-5-phenyl valeric acid	**69%** of 3-hydroxy nonanoic acid, **31%** of 3-hydroxy heptanoic acid

According to the literature, *P. putida* strains, for which the carbon source during fermentation are salts of aromatic acids, produce a polymer consisting of only one type of 3-hydroxy aromatic acids [6]. Due to the nature of the degradation of aliphatic medium length chain fatty acids in the β-oxidation pathway in *P.putida* KT2440, co-polymers composed of 3-hydroxy acids and 3-hydroxy acids shorter by two carbons in acyl moiety are produced [7]. The use of both culture strategies for the strains resulted in a high PHA content in CDW.

Conclusions

The *P.putida* CA3 strain produces poly(3-hydroxy-5-phenylvalerate) homopolymer during fermentation. Pluse-fed flask culture with staged feeding allowed to obtain 57% PHPV content in CDW. *P.putida* KT2440 strain produces heteropolymer during fermentation in a fed-batch reactor, and its content in CDW is 71%.

Acknowledgments

JP acknowledges the fellowship with the project POWR.03.02.00-00-I013/16.

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THE INFLUENCE OF AgNPs AND GO PARTICLES ON THE PROPERTIES OF POLYCAPROLACTONE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 97]

Introduction

According to statistical data, nasal injuries are relatively common as a result of accidents [1,2]. In order to restore the proper functioning of the nose and for aesthetic reasons, surgical reconstructive procedures are performed [3]. Currently, new materials are sought for the production of scaffolds for tissue engineering purposes, which on the one hand will temporarily replace the cartilage lost as a result of trauma, and on the other hand will support and promote the regeneration of the native tissue [4]. Polycaprolactone (PCL) is a commonly known and frequently used biodegradable polymer in tissue engineering [5]. However, this polymer is a hydrophobic material, which may significantly limit cell adhesion to the scaffold surface [6]. Moreover, PCL does not have antibacterial properties, which seem to be crucial in the reconstruction of the upper respiratory tract. The study attempts to modify the PCL polymer. Research on the influence of additives with adsorption (graphene oxide) and antibacterial (silver nanoparticles) properties on the structural and physicochemical properties of composite materials with a PCL matrix was conducted. For this purpose, micro-CT analysis, tests for hardness measurement, roughness measurement and the contact angle measurement were carried out.

Materials and Methods

Polycaprolactone (PCL) (Mn 80 kDa) in a granular form was purchased from Sigma-Aldrich, USA. Silver nanoparticles (AgNPs) in the form of a silver solution (30-35wt.% in triethylene glycol monomethyl ether with a resistivity of 11 μΩ-cm) purchased from Sigma-Aldrich, South Korea. Graphene oxide (GO) in the form of a powder (degree of oxidation: 4-10%, with an edge distribution oxygen functional groups) purchased from Sigma-Aldrich, USA.

Samples were prepared by the technique of melting the polymer. PCL granules with the additive were placed in the melting device heated to a temperature of 110°C, after melting the polymer granule, the ingredients were vigorously mixed with a baguette for 10 minutes. The plasticized polymer was then spread between two laboratory slides to obtain flat, elliptical samples. Two series of samples were obtained: PCL with the addition of AgNPs and PCL with the addition of GO in three different concentrations of modifying additives.

Mechanical properties were characterized by an instrumented hardness tester (InnovaTest Nexus®700).

The roughness measurement was carried out with a roughness tester SRT 220® to quantify the roughness variation on the micro scale. Surface wettability was evaluated by apparent water contact angle measurements. This contact angle was determined by the sessile drop method with an automatic drop shape analysis system OEG OEG®Surftens Universal. To assess the internal geometry of the scaffolds, X-ray microtomography (SkyScan 1172, Bruker, Kontich, Belgium) was performed and the following computer programs: DataViewer®, NRecon®, CTVol® were used.

Results and Discussion

Based on the hardness tests performed, a decrease in the hardness parameter for all samples with an increase in the concentration of the modifying additive was observed.

The roughness measurements showed higher values of roughness for the samples doped with AgNPs and, for each sample, the roughness decreased with an increasing concentration of the modifying additive.

Regardless of the type and concentration of the admixture, it increased the contact angle in comparison to the samples made of pure PCL, and all samples showed a contact angle of less than 90°, and therefore assumed a hydrophilic character.

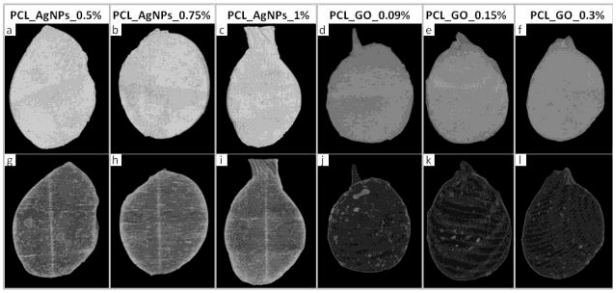

FIG. 1. Micro-CT sample images. The 3D reconstructions of the PCL matrix (a-f), the image of the samples with selected pores (g-l).

In the μCT tests, based on the photos showing selected pores, the highest porosity was found in the PCL_GO_0.09% sample, and the lowest in the PCL_AgNPs_0.75% sample (FIG. 1: g-l).

For each of the samples doped with AgNPs, on the photos showing the selected inclusions, their even distribution was observed, and the higher the concentration of the admixture in the sample, the larger and more numerous aggregates of the additive were formed.

Conclusions

In conclusion, it was shown that the PCL_AgNPs_0.5% sample showed the best physicochemical properties from the point of view of tissue engineering. This sample was characterized by the highest hardness and roughness.

The μCT tests also showed that the PCLAgNPs_0.5% sample was characterized by the most even distribution of admixture particles and the smallest number of aggregates.

Acknowledgments

This work was supported by the The National Centre for Research and Development in the frame of project: "Layered implants for nasal cartilages and bones reconstruction fabricated by 3D printing" TANGO-IV-A/0002/2019.

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BIPHASIC CALCIUM PHOSPHATE BIOCERAMICS DOPED WITH NANOMETALS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 98]

Introduction

Over many years of research, a number of types of materials have been developed and used for various medical purposes, including bone replacement. Calcium phosphate bioceramics (CPs) occupies an important place among them. Its advantages are mainly porosity and high biocompatibility. Because of the similarity in chemical composition of natural apatites in bones, hydroxyapatite and other CPs not irritate the surrounding tissue, does not cause acute or chronic inflammation and stimulate bone repair processes, that enables the creation of chemical bonds at the implant-bone interface. Additionally, CPs biomaterials have exceptional sorption properties and can also intake a variety of isomorphic substitutions in both anionic and cationic subnet, without destroying the basic unit cell. Owing to the above features, it is possible to create new materials for specific applications.

Biphasic calcium phosphate bioceramics (BCP) are commonly used in medicine. Their structure is a combination of stable hydroxyapatite and restorable tricalcium ortho-phosphates. Phases can be mixed in different ratios depending on product's purpose. There is a possibility to make modifications of these materials using ions and nanoparticles. The doping of BCPs with antimicrobial substances may be beneficial in maintaining sterility both at the time of foreign body adaptation and in the postoperative wound healing process [1, 2]

Materials and Methods

One of the main aims of this work was obtaining hydroxyapatite, tricalcium ortho-phosphate and biphasic calcium phosphate bioceramics through applying the wet method. Two sets of reagents were used: $Ca(OH)_2$ and H_3PO_4 or $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$. Physicochemical analysis of prepared materials included determination of molar ratio Ca/P, FT-IR spectroscopy and X-ray diffraction (XRD). The analysis of selected structure parameters such as porosity, pore's volume, weight loss, shrinkage and density were also conducted. Silver nanoparticles (AgNPs) were prepared by chemical reduction of $AgNO_3$ by varied reducing and stabilizing substances. The presence and average particles size of AgNPs were monitored by UV-Vis spectrometry and Dynamic Light Scattering method (DLS). Then, pure BCPs were modified by the "*in situ*" method with silver nanoparticles. Subsequently, preliminary bioactivity tests were performed on all obtained materials in fluids simulating a living organism.

Results and Discussion

As a result of the carried out syntheses, hydroxyapatite (HAp) was obtained with Ca/P molar ratio equal to 1.75. It means that it is a material with excess of calcium. In the case of calcium orthophosphate(V) (TCP), the ratio is 1.71, which is much higher than expected. However, XRD analysis confirmed that only the TCP phase is present in the sample. The occurrence of a higher Ca/P molar ratio may be related to the possibility that some of the phosphate(V) ions were substituted by other ions, such as carbonate CO_3^{2-}, which contributed to the increase of the ratio. In the case of biphasic bioceramics (BCP), the Ca/P molar ratio was determined to be 1.53 and is within the expected range. It is worth noting that the Ca/P molar ratio of bone is reported as 1.70, dentin as 1.62, and enamel as 1.64. This is associated with multiple ion inclusions in these structures [1].

The mass loss in the samples after calcination is at a similar level for HAp and BCP (2.95% and 3.33%, respectively), and is significantly lower in the TCP sample (1.53%).

The synthesis of silver nanoparticles resulted in monodisperse nanoparticles with average size up to 100 nm and narrow size distribution. The location of the absorption maximum of the nanoparticles did not change significantly after about several weeks from the date of preparation of the colloids, indicating the high stability of the nanoparticles over time.

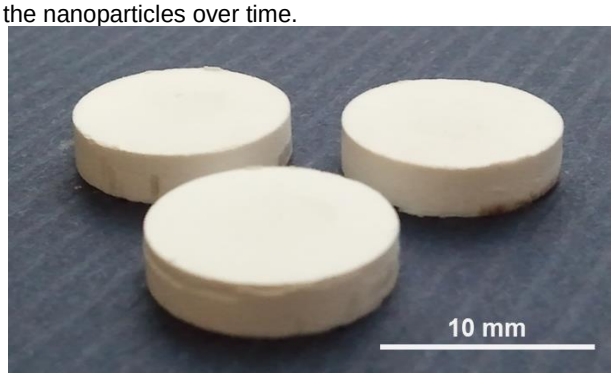

FIG. 1. Test molds of pure BCP before modification with nanometals.

Incubation of nanosilver doped materials under conditions simulating a living organism showed no change in pH and conductivity during the 21-day incubation time. It indicates the stability of the materials and no negative effect on bioactivity.

Conclusions

It can be concluded that development of methods for the preparation of bioactive, metal-modified biphasic calcium phosphate bioceramics can be an important step towards creating a new generation of bioactive materials for applications in biomedicine:

1. The use of different ratios of TCP:HAp allowed us to determine the best ratio for pure and nanosilver doped BCP materials.
2. The Ca/P molar ratio of the obtained BCPs indicates that non-stoichiometric products were obtained.
3. The density and porosity of the investigated materials are within the range desired for implant materials and therefore they can be design as for substitutes for hard tissues.
4. Immersion in simulated fluids does not adversely affect the properties of the tested calcium phosphates, the lack of changes in pH and conductivity during incubation in conditions stimulating a living organism allows their potential use in a living organism.

Acknowledgments

The research was funded by the Department of Chemical Technology and Environmental Analytics's own funds.

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PROPOSAL OF NOVEL ABRASIVE MACHINING METHOD FOR PREPARING THE SURFACE OF PERIARTICULAR TISSUE DURING ORTHOPAEDIC SURGERY OF HIP JOINTS SURFACE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 99]

Introduction

One of the primary methods used during orthopaedic procedures is the surface machining of the cartilage and bone tissue based on drilling, cutting and milling [1]. This type of operation involves thermal energy emission [2], tissue destruction and direct impact on the body [3]. Therefore, the force of the cutting process, temperature, shape and quantity of chips, and the characteristic of the cutting process should be examined. However, the popularity of standardized surgical procedures causes no changes in the processing technology and the availability of specialized surgical equipment. The above study focused on developing a proprietary method of machining the periarticular hip surfaces, focusing on abrasive treatment to remove diseased or damaged tissue. The obtained results provide the first time in literature essential information regarding cartilage and bone tissue abrasion performance.

Materials and Methods

The silicon carbide (95-98% SiC) and brown fused alumina (94,5-97%BFA) grains were used to manufacturing abrasive tools. The polyamide (PA6) rollers with a diameter of 12 mm were covered with mixed styrene-modified epoxy resin with a hardener and grains. Grains with granulation from 90 to 390 um were used. The oscillating movement of the tool in the range of 0.25 to 1 mm in two directions along the shape of the ellipse has been applied. Fragments of pork femoral heads were subjected to machining tests with specific motion kinematics in dry and wet cutting conditions, using a precise UMT Bruker Tribometer equipped with a 2-dimensional force sensor DFM-20 with a measurement range 2 to 200 N and resolution of 10 mN. The cutting temperature was measured with a thermocouple localized in the polyamide roller surface. Primary parameters measured during experiments were normal force F_N, tangential force F_T and penetration depth Δz [mm]. The tool position in the *z, y* and *x* orientation, penetration depth v_z, movement v_x and v_y velocity and Δy, Δx movement ranges were also evaluated. A force sensor in the Z-axis direction monitored a constant load in fourth levels: 5, 10, 15 and 20 N. The friction coefficient was determined experimentally using the tribometer software. The chip forming mechanism was analyzed using a TESCAN Vega 3 scanning electron microscope (SEM) with a secondary electron detector.

Results and Discussion

TABLE 1 presents the friction forces during machining with an abrasive tool. The presence of water allows for lower values of F_{Tmin} and F_{Tavg} for both types of abrasion tools. As a result, the value of the total force increases with the reduction of the grain size. However, the performance of the BFA tool is stable, and the standard deviation is only 0.2 N to the SiC deviation of 0.8 N.

TABLE 1 Maximal, minimal and average tangential force results for different abrasive tool materials and conditions.

Force [N]	BFA wet	SiC wet	BFA dry	SiC dry
F_{Tmax}	14,45	11,20	10,12	11,58
F_{Tmin}	1,65	1,32	2,19	2,68
F_{Tavg}	5,51	5,68	4,80	6,46

The mean value of friction coeffcient μ for cartilage tissue under wet external-jet conditions is μ_{c_ext} = 0,45±0,02 and for cartilage tissue under wet internal-jet (water injected trought polyamide roller) μ_{c_int} = 0,07±0,02. The comparison between bone tissue μ_{b_int} = 0,11±0,02 and cartilage tissue μ_{c_int} shows that erosive machining process is similar.

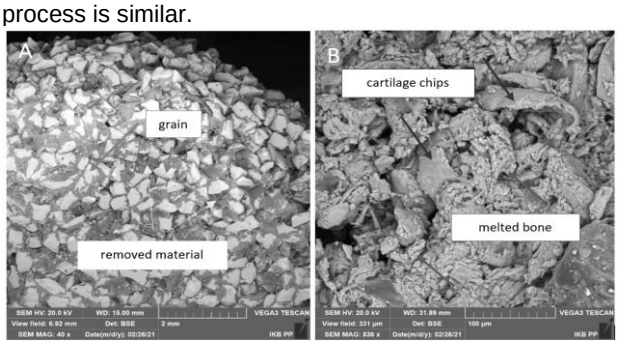

FIG. 1. The effects of machining of cartilage and bone in wet conditions: a) tool surface, b) chips after wet machining.

FIG. 1 is a view of an abrasive surface with remains of bone and cartilage among the grains. In the dry processing of cartilage and bone tissue, the residual cartilage material limited the abrasion mechanism. As a result, the chips did not take any characteristic shapes, creating a slime sticking to the grains. This phenomenon reduced machining efficiency. The separation of tissue fragments from the surface in the form of chips with a continuous, smooth shape was observed during the wet abrasive. During the machining of bone tissue, single fragments of chips were observed. The chip forming stage can be divided into three steps: the single-pass formation of the chip, melting due to multiple tool movements and the final chips exit from the workspace. Regardless of the grain size, this process results in a chip size from 50 to 5 µm. Temperature measurements showed no increase beyond the measuring accuracy range of ±0.4°C.

Conclusions

This machining process method may become a starting point for more extensive research machining and accompanying procedures. In addition, the results highlight the potential of the proposed tool concept in designing new methods for treating periarticular tissues.

Acknowledgements

The research was supported by the subsidy for the maintenance and development of young scientists (SBMN) at the Faculty of Mechanical Engineering of the Poznań University of Technology in 2021.

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RHEOLOGICAL PROPERTIES OF CARBOXYMETHYL CHITOSAN, POLY(N-VINYLPYRROLIDONE) AND THEIR MIXTURES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 100]

Introduction

The polymer mixture consists of two or more polymers are obtained by physical means. Mixtures are made to improve the physico-chemical properties. There are two types of mixtures, heterogeneous and homogeneous. Homogeneous mixtures are miscible. Obtaining a miscible system depends on such parameters as the proportion by weight of polymers, solvent and preparation temperature. [1,2] There are various methods of determining the miscibility, such as viscometric technique, rheological methods, thermal analysis, spectroscopy or scanning electron microscopy [2].
The aim of the study was to determine the rheological properties of the obtained carboxymethyl chitosan/ poly(N-vinylpyrrolidone) mixtures and determination of miscibility using steady shear rheological measurements [3].

Materials and Methods

Chitosan from squid was purchased from POL_AURA. Sodium hydroxide, poly(N-vinylpyrrolidone) (PVP) was supplied Sigma-Aldrich. Sodium chloride was received from POCH S.A. (Avantor, Poland).
Based on the literature, carboxymethyl chitosan (CMCS) was synthesized [4]. Then the polymers were dissolved in various solvents: water or 0.1M sodium chloride. In the next step the polymers were mixed in the following weight ratios: 20/80, 50/50, 80/20. They were stirred on a magnetic stirrer for 24 hours. The steady shear rheological measurements were carried out on a Bohlin Visco 88 rotary viscometer equipped with concentric cylinders at different temperatures (25°-40°C) and shear rates (20-1230s^{-1}). Rheological parameters from the Ostwald de Waele equation were determined and compared [3].

Results and Discussion

Steady shear measurements were performed to evaluate the rheological properties of CMCS, PVP and their mixtures at 25°-40°C. For the carboxymethyl chitosan solutions and its mixtures in distilled water and 0.1M NaCl, the apparent shear viscosity decreases and the shear rate increases, suggesting a shear thinning effect (pseudoplastic nature). In the case of the aqueous solution of CMCS/PVP mixtures, the apparent shear viscosity is higher than that for pure polymer solutions. Furthermore, all polymer solutions are well characterized using a power law model (Ostwald de Waele equation**)**.

Conclusions

The resulting mixtures behave like non-Newtonian fluids. A significant influence of the solvent on the apparent shear viscosity of polymer mixtures and solutions was found. The CMCS/PVP mixtures are miscible systems in distilled water. The CMCS/PVP mixtures in distilled water exhibit a larger apparent viscosity than pure polymers.

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BIOPOLYMER MICROSPHERES MODIFIED WITH MAGNESIUM AND ZINC IONS FOR TISSUE ENGINEERING APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 101]

Introduction

One of the problems still faced by various tissue engineering scaffolds is the lack of proper vasculature that would aid regeneration of tissue defects. Formation of a network of blood vessels through angiogenesis is one of the most effective ways to sustain healthy cells metabolism by delivering nutrients, signalling factors and removing waste. This is fundamental for successful tissue regeneration. Among many proangiogenic factors, metal ions, such as magnesium, strontium, copper, or zinc are particularly interesting due to their additional therapeutic activities [1]. They can be used in various systems. However, biopolymer-based carriers seem to be one of the most versatile and possess all the advantages of being naturally-derived, e.g. biocompatibility, sustainability, high availability, relatively low cost.
The aim of the study was to design and characterize polysaccharide-based systems for future use for angiogenesis enhancement in tissue engineering applications. Magnesium and zinc ions were selected as active molecules for surface modification of chitosan microspheres.

Materials and Methods

Chitosan microspheres were fabricated by dropping chitosan solution (1.5% CS, Chitosan 85/1500, Heppe Medical Chitosan GmbH (HMC+) in 1% acetic acid) into a constantly stirred gelling bath (5% sodium tripolyphosphate (TPP), 0.15M NaCl, 2% Span80) through a 0.7 mm needle. A volume ratio of CS solution to the gelling solution was set at 1:10. The formed beads were crosslinked for 48 h, subsequently centrifuged and washed with isopropanol, ethanol, and distilled water. To immobilize magnesium and zinc ions on the surface of CS carriers, microspheres were immersed in an appropriate ion solution (500 mg/L of Mg^{2+} or Zn^{2+}) for 48h under constant stirring, drained and washed with distilled water. Prior to characterization, all CS-based beads were frozen and freeze-dried. Microstructure (digital microscopy, SEM-EDS), chemical stability (weight loss, water absorption), structure (FTIR) and ion release (AAS - Atomic Absorption Spectroscopy, air-acetylane flame) were evaluated.

Results and Discussion

The parameters of the fabrication method were carefully optimized considering concentration of polymer solution, needle diameter, composition of a gelling solution, crosslinking time, etc. All of the above affect the final properties of the microspheres. The obtained chitosan beads were round and homogenous with a diameter of 904±94 μm (FIG. 1). After modification with Mg^{2+} and Zn^{2+}, slight morphology changes were observed as a result of exposure of hydrogel spheres to water-based ion solution. Also, the diameters increased to 1080±125 μm and 1138±79 μm for CS-Mg and CS-Zn, respectively. The presence of magnesium and zinc on the surface of the microspheres was verified by the SEM-EDS analysis.

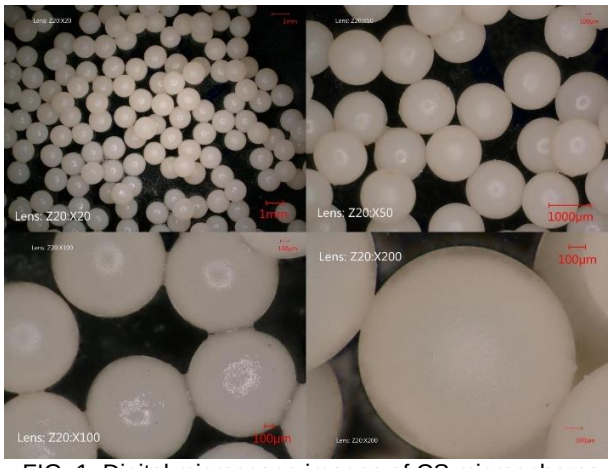

FIG. 1. Digital microscope images of CS microspheres (mag: x20, x50, x100, x200).

Chitosan was crosslinked in the TPP-based solution. FTIR spectra (FIG. 2) confirmed that phosphate groups were present in the final material. As expected, the crosslinking effect occurred due to the interactions between negatively charged PO^- groups of TPP and positively charged NH_3^+ groups of CS. Changes in the spectra related to Mg^{2+} and Zn^{2+} modifications were also visible.

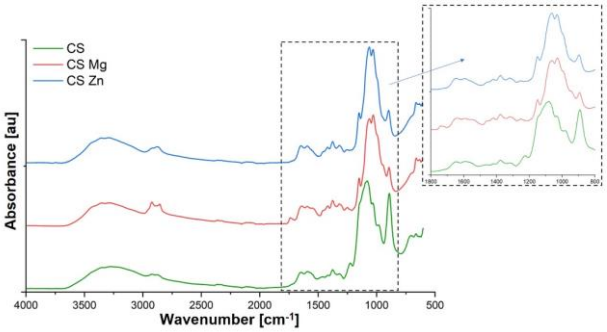

FIG. 2. Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy spectra of unmodified (CS) and ion-modified samples (CS-Mg and CS-Zn).

AAS analysis showed that release kinetics differed for both ions. Higher final concentration was achieved for magnesium-modified CS beads. The release rate and kinetics can be modified.

Conclusions

Chitosan microspheres can be used as carriers of magnesium and zinc ions, known for their proangiogenic activity. Further, detailed studies are ongoing.

Acknowledgments

This work was supported from the subsidy of the Ministry of Education and Science for the AGH University of Science and Technology in Kraków (Project No 16.16.160.557).

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EFFECT OF CHEMICAL TREATMENT OF POLYLACTIDE ON PROTEIN ADSORPTION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 102]

Introduction

The biomaterials surface plays a crucial role in protein adsorption, cells adhesion and biological tissue response. One of the main surface parameters is its hydrophobic/ hydrophilic character. Hydrophilic properties foster cells adhesion and proliferation [1]. This feature is important for bone regeneration. On the other hand, in cardio-surgery, hydrophobic surfaces counteract platelets aggregation [2]. Protein adsorption is the first stage of the biomaterial-tissue interaction and surface properties first decide on the type of the protein attached. The composition and structure of the adsorbed protein layer is considered one of the main factors determining the type of interaction between cells and biomaterials [3]. Albumin is an inhibitor of thrombotic processes, that is why it plays a key role in the case of biomaterials intended for contact with blood [2].
In this study, chemical treatment of polylactide (PLA) was approached. It was analysed how the changes in the surface wettability affect the albumin adhesion. The impact of a PLA manufacturing method (injection-moulding and casting from different solutions) on the surface wettability was also evaluated.

Materials and Methods

Polylactide (PLA) Ingeo 3051D, NatureWorks®LLC was used. Injection-moulding was performed by using the screw injection moulding machine (MULTIPLAS) at 160°C. The second group of the samples were obtained by casting from the solution of PLA in CH_2Cl_2 (POCH) in proportion 1g/50ml and the third group from the solution of PLA in 1.4-dioxane (POCH), 1g/40ml. All the samples were shaped as round plates with a diameter of 1cm. One group of the PLA samples obtained from 1.4-dioxane solution were treated in 0.1M NaOH (POCH) and the second group in 0.1M HCl (POCH) for 20 sec. Next, the samples were rinsed with distilled water. In the next step, the samples were incubated in the albumin solution (albumin from chicken egg white, Sigma Aldrich) for 20min at 37°C, 1 sample per 2ml of the albumin solution and 8ml of the culture medium (Lonza, USA). The albumin solution was prepared by mixing 1g of albumin in 10ml of water. 4 samples were tested in every group. Contact angle was measured 10 times for every sample by using DSA10-Mk2, Krüss. Standard error was applied. The albumin was identified on the basis of infrared spectroscopy in ATR technique by using the BIO-RAD FTS 3000 Excalibur Series (PIKE) spectrometer. The MIRacle ATR diamond with ZnSe optics was used.

Results and Discussion

The influence of the manufacturing method on the surface wettability was observed (TABLE 1). PLA obtained from the 1.4-dioxane solution is characterised by more hydrophobic character (contact angle 90.7°) than PLA from the CH_2Cl_2 solution or the injected PLA. The contact angle measured for the injected PLA and for the CH_2Cl_2-based PLA is 72.2° and 76.5° respectively and these values are comparable to the literature characteristic of polylactide [2]. PLA obtained from 1.4-dioxane solution was treated in HCl and NaOH and next incubated in albumin because of the hydrophobic surface which is important for cardiovascular and cardiosurgical application. The treatment in HCl increased the contact angle to 107.3°. The NaOH treatment caused only slight changes of the surface wettability that are within the limits of statistical error. The obtained results prove the albumin adhesion on all the samples (untreated and treated in HCl and NaOH). The albumin adhesion is indicated by the strong hydrophilic character of the samples. The measured values of contact angles are approximately equal to 20°. The high dispersion of results after the albumin adhesion reveals no statistically significant differences between these samples. This can be related with the uneven albumin distribution on the PLA surface. The ATR spectra show that the amount of attached protein is different for the tested samples (FIG. 1). The bands connected with amid I (1655 cm^{-1}) and amid II (1540 cm^{-1}) are the most intensive for the spectrum of PLA treated in HCl after its incubation in albumin solution. In the spectra of the untreated PLA and the PLA treated in NaOH the intensity of bands connected with albumin is low, however the presence of these bands confirms the albumin adhesion. The mechanism of albumin adhesion on PLA was analysed by Kiss et al. [2]. The other bands observed on the spectra are typical for polylactide.

TABLE 1. Contact angle of samples.

SAMPLE	CONTACT ANGLE [°]
PLA $_{injected}$	72.2 ± 2.2
PLA $_{CH2Cl2}$	76.5 ± 2.7
PLA $_{dioxane}$	90.7 ± 2.5
PLA_{HCl}	107.3 ± 6.3
PLA_{NaOH}	94.8 ± 3.8
PLA with albumin	24.8 ± 10.1
PLA_{HCl} with albumin	22.5 ± 4.5
PLA_{NaOH} with albumin	16.4 ± 5.1

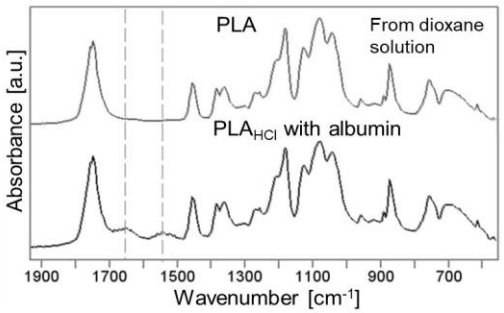

FIG. 1. ATR spectra of selected PLA samples.

Conclusions

The influence of the PLA solvent on the surface wettability was observed. The PLA dissolution in 1.4-dioxane resulted in the more hydrophobic surface.
The PLA treatment in HCl increased the value of contact angle and affected the higher adhesion of albumin.

Acknowledgments

This work was supported from the subsidy of the Ministry of Education and Science for the AGH University of Science and Technology in Kraków (Project No 16.16.160.557).

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ANTIBACTERIAL PROPERTIES OF CARBON FIBERS WITH LAYER DEPOSITED BY MAGNETRON TECHNIQUE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 103]

Introduction

A new look at carbon materials with a future in medicine is carbon nanofibers (CNF) or carbon fibers (CF) with controlled surface properties. Due to the fibrous form (nano)fibers can provide an attractive substrate to aid in the regeneration of many challenging tissues including neural and cartilage due to their microstructure that mimics the geometry of the extracellular matrix [1-2]. A well-known approach in the environmental protection is to use carbon fibers in the active form called activated carbon fibers (ACF) in which porous, highly absorbing surface exhibits bactericidal properties. These fibers are often subsidized with biocidal agents such as silver or zinc.

Carbon fibers with activated surface are the most promising absorbents (supports) characterized specific and selective capabilities for interacting with biologically active molecules and organisms. Fibers containing an ultra-dispersed metal phase represent a new type of absorbent which action is based on affinity for proteins and heavy-metal ions e.g.; Ag, Zn, Cu, Ni which induct special interaction with bacteria. In many instances, such fibers are called nanostructured composites [3].

In our research we propose to use classical low modulus carbon fibers, commonly used in medicine, as a carrier of active layer of bactericidal character. The carbon fibers (CF) were subjected to appropriate physicochemical treatment i.e. magnetron sputtering and covered with a layer of titanium, copper and zinc. A CF/Zn was used as a positive reference for CF/Ti and CF/Cu fibers investigated. Their antibacterial activity against Gram-positive and Gram-negative bacterial cultures was investigated.

Materials and Methods

A two-step thermal conversion process was used for the thermal treatment of polyacrylonitrile (PAN, Sigma-Aldrich) polymer fibers. The base material for this process was a polyacrylonitrile nonwoven with a surface density of 120 g/m^2. The first step of thermal conversion was oxidation (250^{o}C/air) and the second step was low-temperature carbonization (970^{o}C/nitrogen). Fiber morphology was observed using scanning electron microscope (Nova NanoSEM, FEI). The samples were modified using the DC magnetron sputtering system produced by P.P.H. Jolex s.c. (Czestochowa, Poland). The modifying metallic layers were deposited in a protective atmosphere using copper, titanium and zinc targets. The presence of the layer and its uniform distribution on the fiber surface were verified by SEM/EDS. The effect of the layer on physicochemical properties such as contact angle and surface free energy was evaluated with a 25 Kruss goniometer.

All materials were subjected to antimicrobial testing using the Kribby-Muller diffusion method. Morphology of Gram-positive and Gram-negative bacterial cultures contacted with fibrous substrate modified with a layer of copper, titanium and zinc was also examined.

Results and Discussion

The experimental results show that the process of thermal conversion of the polymer fiber with PAN to carbon fibers induces a fiber shrinkage of 6%. Thus, the diameter of the carbon fiber comparing to the diameter of the precursor polymer fiber decreases from 15 to 11 μm. The carbon fibers exhibit a significant decrease in wettability relative to that of the polymer nonwoven. Magnetron sputtering leads to a metallic layer on the source-exposed side of the nonwoven. The layer has a homogeneous character on the outer fibers. Its presence determines the increase of wettability from 30^o for carbon nonwoven to 50-62^o for carbon nonwoven with titanium and copper layer, respectively. All metallic layers show bacteriostatic effect and the nonwoven fabrics with copper layer also show bactericidal effect regardless of the type of strain used in the tests (FIG. 1).

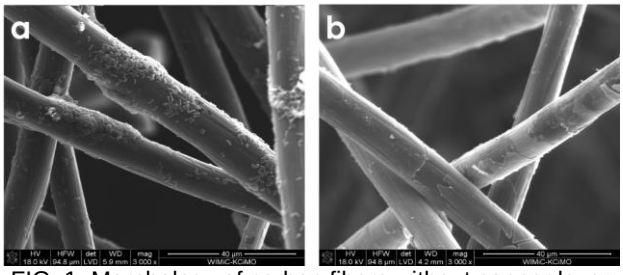

FIG. 1. Morphology of carbon fibers without copper layer (a) and with copper layer (b) after exposure to *E.coli.*

Conclusions

Carbon fibers formed by thermal conversion of PAN can be modified by magnetron sputtering. The resulting metallic layer is only external, and its thickness depends on the process conditions. The presence of the layer increases the contact angle and has an antibacterial activity.

Acknowledgments

The work was supported by the National Science Center – Poland: Project no: UMO-2018/31/B/ST8/02418

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SURFACE FUNCTIONALIZATION OF MWCNTS TOWARDS BIOMATERIALS APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 104]

Introduction

Since raw carbon nanotubes (CNTs) are entirely hydrophobic, their applications as biomaterials require surface functionalization. Such modifications are one of the main strategies to improve the dispersibility in the polar solvent of choice. Additionally, the presence of functional groups enables the anchoring of molecules to carbon surfaces, including attachment of bioactive compounds. Recently, the topic is intensively explored. Functionalization can either be covalent or noncovalent and the strategy applied depends mostly on the target application.

The chemical nature of introduced functional groups, their surface concentration and location have a significant impact on the properties of carbon nanotubes. For such materials, oxygen-containing functional groups are most often introduced. These include the carboxyl, hydroxyl and epoxy groups. Polar oxygen groups (due to the electronegativity difference between carbon and oxygen atoms affect mostly the wettability of the carbon surface and its electron-donor properties, which are often taken into account when designing carbon materials for specific applications, especially in the context of biomaterials [1].

One of the problems with applications of carbonaceous materials is the difficulty in obtaining good quality suspensions in water and other polar solvents. Oxygen-containing functional groups improve the wettability and transform hydrophobic surfaces into hydrophilic what is considered a key modification since improving the biocompatibility of carbon-based implantable materials. The introduction of functional groups substantially changes the electronic properties of carbon materials. Indeed, as reported elsewhere the work function was indicated as a suitable parameter to assess the quality of biomaterials' surface.

The work aimed to investigate the effect of functionalization of multi-wall carbon nanotubes using the wet chemistry method and oxygen plasma treatment on the properties of the carbon materials. The effect of functionalization was evaluated in terms of the quality of the obtained suspensions in polar solvents monitoring the sedimentation of carbon materials in times.

Materials and Methods

MWCNTs used in the study were purchase from NanoAmor (>98%, inner diameter 20-30 nm). The materials were functionalized either using wet chemical methods or plasma treatment (see details in TABLE 1) [2]. The investigated materials were characterized with the use of Raman Spectroscopy (structural changes) and work function measurements (surface changes).

Results and Discussion

Raman spectroscopy revealed significant differences in the intensity ratio of the maxima (ID/IG). The comparison between unmodified and modified MWCNTs (wet methods and oxygen plasma) revealed an increase in the ID/IG ratio after modifications. Thus, the obtained spectroscopic profiles indicated defects introduction, which can be associated with breaking of the C-C bonds upon formation of oxygen functional groups.

TABLE 1. The list of investigated samples together with the applied modification parameters.

samples	**wet chemical methods**
H16	16 h H_2O_2 + H_2SO_4
K8	8 h H_2SO_4 + HNO_3 (3:1)
K16 ½	16 h H_2SO_4 + HNO_3 (1.5:1)
K16	16 h H_2SO_4 + HNO_3 (3:1)
samples	**plasma functionalization**
MWCNTs 0.1 min	0,1 min., 0,2 mbar, 60 W
MWCNTs 5 min	5 min., 0,2 mbar, 60 W
MWCNTs 20 min	20 min., 0,2 mbar, 60 W
MWCNTs 60 min	60 min., 0,2 mbar, 60 W

Electrodonor properties of MWCNTs modified with the wet methods and by oxygen plasma treatment were measured with the use of Kelvin Probe. The results show that oxygen functional groups have a great influence on the electronic properties of MWCNTs. The unmodified material work function value was 4.58 eV. Both modifications significantly increase the work function values, for wet methods the ΔΦ was in the range of 0.1-0.3 eV (FIG. 1), while for oxygen plasma-treated samples the increase was substantially larger 0.7-1.1 eV.

In order to compare the stability of the modifications performed with the use of wet methods and plasma, suspensions of carbon materials in water, ethanol and acetone were prepared and their sedimentations were followed for 36 days. It was found that modifications with wet chemical methods were stable for 30 – 60 days while plasma modifications diminish within 24h.

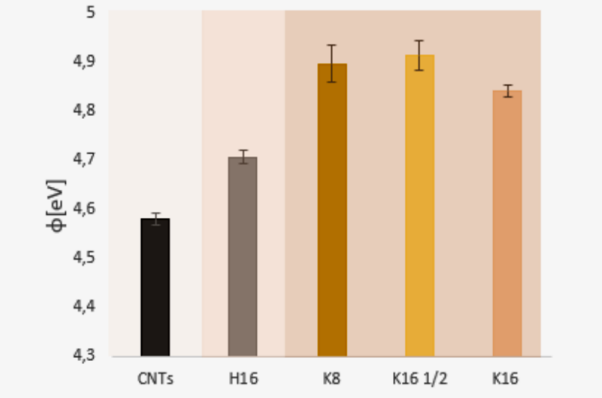

FIG. 1. The changes in work function values of the investigated carbon nanotubes modified with wet chemical methods (CNTs: unmodified reference sample, for other labels see TABLE 1).

Conclusions

The obtained results clearly show the necessity of careful selection of the functionalization method for specific applications. Moreover, the modification process parameters precisely adjusted for both of the applied methods (for wet methods: modification time and type of oxidant, for plasma method: modification time, partial pressure gas, generator power) are of key importance.

Acknowledgments

This study was funded by Polish National Science Centre project, award number 2020/37/B/ST5/03451

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HYDROGELS FOR 3D BIOPRINTING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 105]

Introduction

The biofabrication of three-dimensional (3D) biomimetic tissue analogs, which accurately mimic the properties of native tissue, has enormous potential for research in organ physiology, pathology, cancer research and regenerative medicine. A significant impediment for the development of therapies is the shortage of available tissues and the inability to sustain mature tissue cultures. Bioprinting is one of the dynamically rising techniques to biofabricate biomimetic tissues, based on in vivo development (cellular self-assembly and -organization) [2,3].

One of the strategies to improve the bulk and surface properties of biomaterials is the incorporation of bioactive substances. Although for hydrogels a number of nanoparticle-incorporation strategies were investigated, they are mostly related to carbon, metals, and oxides. Here, we propose a new approach for embedding bioactive substances nanoparticles via a one-step sonochemical method, i.e. simultaneous formation and anchoring. The proposed approach is versatile and opens a plethora of possibilities to form nanoparticles of various bioactive substances. This makes a possibility for releasing therapeutic agents from the generated nanoparticles with controlled kinetics. It results from the fact that the size, morphology, and incorporation depth of nanoparticles can be easily tuned by adjusting the parameters of the sonochemical process.

The aim of the study was to optimize the sonochemical parameters in order to obtain homogenous dispersion of nanoparticles in the hydrogel without damaging the chemical structure of the material.

Materials and Methods

Methacrylamide-modified gelatin (GelMA) was prepared following the protocol [3], in the studies, 10 w/v% GelMA hydrogel in PBS was used.

Metal and oxide nanoparticles of Au (5 and 35 nm) and Fe_3O_4 (5 nm and 30 nm) were incorporated in the GelMA matrix. For the dispersion of nanoparticles in the prepared GelMA solution Ultrasonicator Sonics Vibracell was used while the following parameters were changed: sonication time 10-30s, mode pulse or continuous, amplitude 20-40%. After the synthesis, the obtained materials were lyophilized and characterized with the use of TG/DTA, ATR-IR spectroscopy, and Scanning Electron Microscopy.

Results and Discussion

The materials were carefully evaluated after each set of sonochemical treatments in order to check the GelMA stability. For the applied parameters none of them caused damage to the GelMA chemical structure which was confirmed with TG/DTA (no changes in melting point) and ATR-IR spectroscopy (no changes in the molecule fingerprint, see spectra in FIG. 1). However, the amplitude above 30% causes overheating of the samples and their foaming especially the latter one counteracts the crosslinking process. As a result, the optimal sonochemical parameters (10s of continuous mode, 30% amplitude) were somehow the compromise between homogenous dispersion of the nanoparticles in the GelMA and its macroscopic morphology after sonochemical treatment.

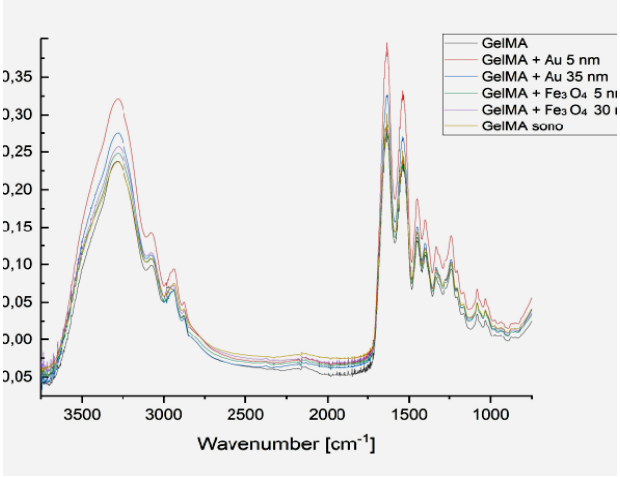

FIG. 1. Representative ATR-IR spectra of parent hydrogel GelMA as well as nanoparticles-functionalized GelMA treated sonochemically (10s of continuous mode, 30% amplitude).

The resultant morphologies of nanoparticles-functionalized GelMA were then observed with the use of SEM (FIG. 2).

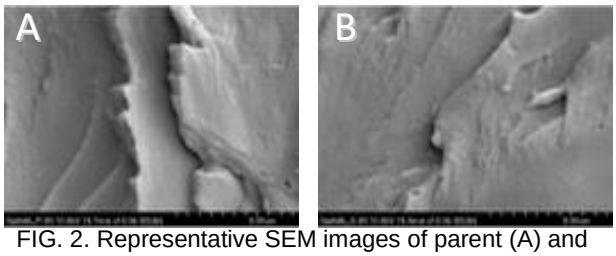

FIG. 2. Representative SEM images of parent (A) and nanoparticles-functionalized (B) GelMA hydrogels.

Conclusions

The obtained results showed that the optimized parameters of ultrasonic functionalisation of GelMA do not change the native chemical structure of hydrogel and incorporated bioactive substances. In broader context the sonochemical functionalization can be used as a versatile method for bioinks fabrication with adjusted both realogical and biological properties.

Acknowledgments

This study was financed by the CELSA/20/020 SMARt biomaterials for bioprinting of vascularized TISsues (SMARTIS).

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OXYGEN PLASMA MODIFICATION OF POLYESTER-BASED POLYURETHANE SURFACES: STABILITY OF SURFACE FUNCTIONALITIES IN TIME

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[ENGINEERING OF BIOMATERIALS 163 (2021) 106]

Introduction

Polyurethanes are a large family of polymers widely used in medical devices with one common characteristic of the presence of urethane linkages along the large molecular chains. The urethane linkages are formed by the reaction of isocyanates (-NCO) and alcohols (-OH) and in general consist of only a small component of the total chain, with the greatest number of linkages contributed by the macroglycol. Polyurethanes are among the best choices for biomedical applications because of their tunable properties such as durability, elasticity and fatigue resistance, connected with their segmented polymeric character. Thus, polyurethane can be tailored to meet the desired properties for a wide range of applications.

Owing to their versatility of chemical composition, polyurethanes are now the highest-performing biomedical-grade elastomers. For biomaterial applications, the interaction of a material's surface with cells is of critical importance. The interaction strongly depends on the physicochemical properties of the surface, such as its hydrophilicity, roughness, or presence of functional groups, microstructure, and mechanical properties. Such properties determine cell attachment, as well as cell spreading behavior, proliferation, and differentiation.

The surface characteristics of a material can be adjusted to a specific application by surface modification techniques, without changing the material bulk properties. The capacity of polyurethanes to undergo modifications increases their suitability for biomedical applications. The functionalization can be obtained with the use of plasma treatment by the generation of surface functional groups. The superiority of the proposed method is based on its simplicity, efficiency, and environmental friendliness.

Materials and Methods

The commercially available aromatic polyester polyurethane samples provided by American Polyfilm, Inc were modified using oxygen plasma using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany) at variable parameters (oxygen partial pressure, generator power, modification time). The changes within the surface were followed by contact angle measurements, using a Surftens universal instrument (OEG GmbH). Static contact angles of water were calculated using Surftens 4.3 — windows image processing software for digital images for the determination of contact angles and surface tension. The chemical surface composition of the polyurethane samples was examined with the use of XPS (SES R4000, Gammadata Scienta).

Results and Discussion

One of the most common techniques for improving biocompatibility of polymeric surfaces is oxygen plasma treatment. The most important issue is to adjust plasma parameters for particular polymeric material and its application. Detailed characterization of the oxygen plasma-treated surfaces is crucial because the biological moieties are rigorously sensitive to the geometrical and chemical factors of the material surface.

The introduction of oxygen functional groups has a significant influence on the wettability of the investigated material. Unmodified polyester polyurethane remains hydrophobic, with a water contact angle slightly above 90°. In this work, several oxygen plasma modification parameters were tested (pressure 0.12, 0.2, 0.3 mbar; plasma generator power 25, 50, 75, 100 W, and plasma treatment time 6s -10 min). It was revealed that the strongest impact on surface modification has the oxygen partial pressure in the plasma chamber (FIG. 1A). A fully wettable surface was obtained for the lowest modification pressure (0,12 mbar). A slight modification was observed for 0,3 mbar and even after a long modification time (10 min) the water contact angle does not exceed 50°. It was also revealed that plasma generator power has no significant effect in the investigated range.

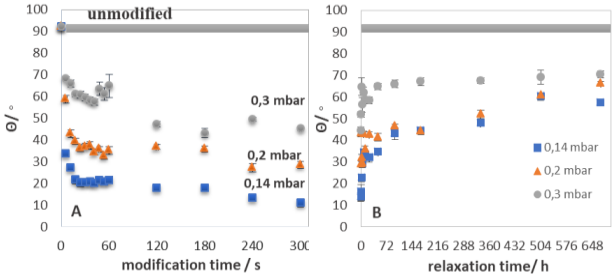

FIG. 1. Experimental water contact angles (θ) for different gas partial pressure (A), and the corresponding kinetic curves of plasma relaxation of activated polyurethane surfaces (B).

To identify the chemical nature of the generated surface functional groups on the polyurethane were investigated with the use of XPS. The results revealed the significant changes between oxygen plasma modified and unmodified surfaces. The XPS spectra (C1s) for both surfaces were deconvoluted, and the characteristic binding energies for oxygen-containing groups were assigned (C=O, C-O, COOH, OH).

The oxygen plasma treatment leads to fully wettable polyurethane surfaces. The modification effect is, however, not stable in time, and the relaxation kinetic curves of hydrophobicity are presented in FIG. 1B. The increase of water contact angle in time was observed for 2 weeks after plasma treatment from ~10 to 60°, yet, never reached the initial value for the unmodified sample.

Conclusions

The oxygen plasma modification on polyurethane surfaces is an efficient technique for increasing hydrophilicity and has a significant impact on biocompatibility. The presented studies revealed oxygen partial pressure as a crucial parameter for polyester-based polyurethane modification. The oxygen-containing groups incorporated in the polyurethane surface provide a suitable platform for further modification. Since the assumptions for the functionalization procedure are of a general nature, the obtained results can be easily extended for other plasma feed gases and polymeric materials.

Acknowledgments

This work was supported by the National Science Centre, Grant 2019/35/D/ST5/03107

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MODIFIED Ni-Ti WIRE FOR MEDICAL MESH APPLICATION

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[ENGINEERING OF BIOMATERIALS 163 (2021) 107]

Introduction

Shape Memory Alloys (SMA) are certain metallic materials which after deformation, regain their original shape. This phenomenon is connected with reversible thermoelastic martensitic transformation. Shape memory effect can be induced by external stress, temperature and/or magnetic field [1]. Ni-Ti alloys (Nitinol) are the most widely used shape memory alloys in medicine, due to their exceptional mechanical properties, high corrosion resistance and biocompatibility. Implants made of Ni-Ti wires are commonly used in cardiac surgery e.g.: blood vessels stents, amplatzer septal occluders, clots removal devices, embolic protection devices [2]. In most cases, after expansion, implant keeps the programmed shape and stays at fixation place. However, there are cases when healed/supported tissue or organ changes its' shape in time eg.: during cardiac muscle contractions [3]. Thus, stent or implant should follows these changes. Moreover, its properties should comply with requirements of cyclic repeatable deformation with no fatigue effect. For this purpose, another unique feature of SMA can be exploited – superelasticity.
The present work provides a brief overview of preliminary evaluation and verification of commercially available Ni-Ti wire properties for use in braiding cardiac implants designed for work in cyclic deformation conditions connected with contracting myocardium.

Materials and Methods

Commercially available Ni-Ti wire with 0,5 mm in diameter was used (BHH Mikrohuta sp. z o.o., Valbruna Group, Dąbrowa Górnicza, Poland). First, the temperature range of the reversible martensitic transformation was determined using a DSC differential scanning calorimeter (Metler Toledo DSC 1). Then the presence of superelasticity effect was verified under uniaxial tension test (Zwick-Roell 1445 RetroLine). Test speed was 1 mm/s. Uniaxial tension test was cyclically repeated on the same sample and deformation was increased by 0,5% each time respectively. The test was aborted when first signs of elastic deformation of inducted martensite appeared.

Results and Discussion

FIG. 1 shows the thermograms measured for the tested wire. In order to confirm the repeatability of the reversibility of the martensitic transformation, two thermal cycles were measured. Comparison of thermograms indicates the repeatability of the martensitic transformation occurrence. It appears in the temperature range from -8°C to 20°C. The temperature of the end of reverse martensitic temperature is about 17 degrees lower than that one of the human body. This difference meets the requirements for the safe use of the wire for a medical implant.

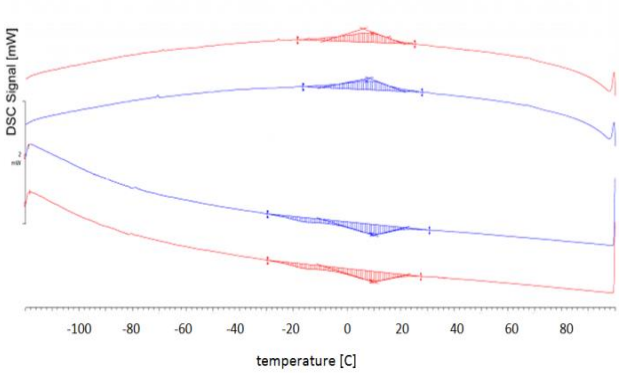

FIG. 1. DSC cooling/heating curves measured for studied wire.

Such conditions guarantee the occurrence of the superelastic phenomenon. Its presence was confirmed in the cyclic tensile test (FIG. 2). The nature of the measured curves indicates that the alloy is in its parent phase under zero stress, as confirmed by the DSC curves. The introduction of external tensile stress - induced martensite. Consequently, a change of shape was observed. After its unloading, the wire returned to its original size, undergoing a reverse martensitic transformation. In the first deformation cycle, up to 0.5%, the martensite-inducing critical stress was 570 MPa, while in the last one, its value decreased to 509 MPa. The stress-inducing reverse martensitic transformation was about 51 MPa.

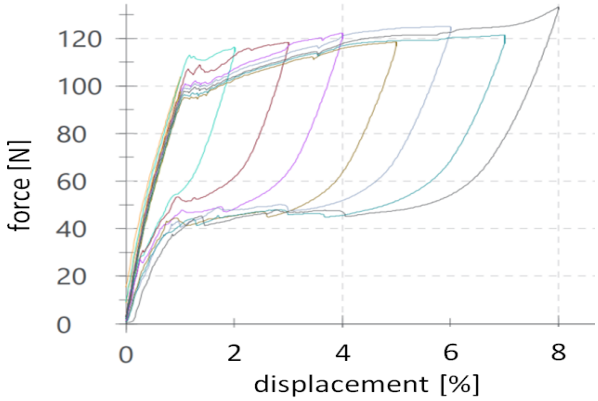

FIG. 2. Cyclic stress-strain curves measured for Ni-Ti wire.

Conclusions

The obtained results, both from thermal analysis and mechanical testing, proved that tested Ni-Ti wire reveals the repeatable martensitic transformation in the temperature range from -8°C to 20°C and the occurrence of superelasticity up to 8%. Such characteristics are desired for further development of tailored braided cardiac implant designed for working in cyclic repeatable deformation conditions.

Acknowledgments

The authors acknowledge financial support from *Regionalny Program Operacyjny Województwa Małopolskiego 2014-2020* (RPMP.01.02.01-12-0059/19) and CardioCare Sp. z o.o.

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ELASTIC, ELECTRICALLY CONDUCTIVE, CYTOCOMPATIBLE AND BIOPRINTABLE COMPOSITES

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[ENGINEERING OF BIOMATERIALS 163 (2021) 108]

Introduction

Elastic materials which are electrically conductive and, at the same time – cytocompatible and formable through 3D printing techniques are of great interest in the today's medicine [1]. First of all, such materials can be used to fabricate wearable sensors for real-life monitoring of biological signals [2,3]. Second of all, they can be used in designing new type of *in vitro* culturing chambers that introduce exogenous signals (such as electrical stimulation, ES) to produce desired cellular response. As numerous studies suggest, exogenous ES can stimulate synthesis and maturation of the extracellular matrix, enhance proliferation and maturation of cells, or even induce differentiation of stem cells into certain lineages [4-8].

As such, it seems that designing novel types of electrically conductive, cytocompatible, easily formable materials is of great importance. Specifically, designing novel ES stimulation chambers which combine an ease of use with the ability to benefit from the specifically designed, biofunctional scaffolds, seems like a stepping stone in the progress of tissue engineering. This is true especially for the tissues with low regenerative potential – muscle tissue (including heart) and neural tissue.

The aim of this study was to optimize the composition and the fabrication procedure for the obtainment of electrically conductive, cytocompatible and bioprintable elastic materials.

Materials and Methods

2-component polydimethylsiloxane (PDMS) was purchased from Dow-Corning, 2 types of mutli-walled carbon nanotubes (CNTs): 3100 and 3150 were supplied by Nanocyl Company. Trimethylsiloxy terminated PDMS was bought from Gelest. The composites with 1% wt. of each type of CNTs were fabricated in accordance to protocol established by Kim, et al. [2], with small modifications.

Dispersion of the CNTs within the matrix was evaluated via Keyence digital microscope (VHX-900F), chemical composition was analyzed through FTIR-ATR spectroscopy (Tensor 27, Bruker). Inspekt Table universal testing machine (Hegewald – Peschke) revealed the effect the CNTs had on the mechanical properties of the PDMS. Ossila 4-point probe was used to evaluate the sheet resistance of the material.

Cytocompatibility of the as-obtained materials was tested through the preparation of liquid extracts, in accordance with ISO 10993-5 standard [9]. Empty cell well served as a blank, and pure PDMS was used as a negative control. The tests were conducted on HEK 293 cells, cytotoxicity/cytocompatibility was established through MTT and LDHA analyses.

Finally, bioprintability of the obtained materials was analyzed via Cellink BioX device.

Results and Conclusion

The undertaken protocol allowed for fabrication of homogenous materials. Presence of CNTs granted the PDMS with electrical conductivity and enhanced mechanical properties.

Tests with cells revealed that the obtained nanocomposites are cytocompatible.

Finally, a good bioprintability of the material was found. The obtained results indicate that the fabricated ink is a promising material for the fabrication of cytocompatible & electrically conductive, 3D-printed materials.

Acknowledgments

This study has been supported by the National Centre for Research and Development under grant no. LIDER/7/0020/L-11/19/NCBR/2020.

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COLLAGEN EXTRACTION FROM THE MICE TAIL AND ITS SUCCESSIVE ELECTROSPINNING

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[ENGINEERING OF BIOMATERIALS 163 (2021) 109]

Introduction

Collagen is one of the main constituents of the extracellular matrix of mammalian tissues, serving as a natural scaffold that maintains the proper shape and mechanical properties of various tissues types [1-3].
As such, its applicability in various fields of medicine is of high importance, especially when tissue engineering is regarded. While this material is abundant in various tissue types, its proper extraction, being able to maintain its bioactivity and biofunctionality can be challenging. Then, its further processing into the proper shape and morphology can also become cumbersome.
Among different shapes that are regarded for its biomedical applications, the fibrous morphology is certainly one of the most popular, as it guarantees the highest biomimetism, high-surface-to volume ratio and enhanced mechanical properties [4].
The aim of this study was to conduct isolation of the collagen fibres from the mice tails in such a manner, that the material's native conformation is maintained. Another goal was to optimize the electrospinning conditions of the collagen so that the materials of fibrous morphology, insoluble in water can be obtained.

Materials and Methods

Mice tails were harvested from the post-experimental animals, in accordance with the 3R rule, through the courtesy of the UJ Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland. The tails were processed in accordance with the protocol established by Rajan et.al. [5] – with exception that the process was conducted only until lyophilized sponges at step 25 were obtained. By this means, we obtained a mixture of different collagen types, which is suitable for the successive electrospinning. The materials were characterized via Keyence digital microscope (VHX-900F) and FTIR-ATR spectroscopy (Tensor 27, Bruker).
The as-obtained collagen was then dissolved using various solvents, including PBS, ethanol, and acetic acid in order to optimize the solvent composition for the electrospinning (SKE EF3000). The as-obtained fibres were tested for their chemical and morphological properties, with an aim to analyse the impact the process had on the materials' initial properties.

Results and Conclusions

We found that it is possible to extract the collagen fibres from the mice tail, with similar efficiency as the one reported for the rat tails. The materials maintained their native conformation and were soluble in some of the tested solvent compositions.
While the ES process was found to be challenging, we were still able to produce the fibrous products with satisfactory properties.

Acknowledgments

This study has been supported by the National Science Center, Poland, under grant no. UMO-2017/24/C/ST8/00400.
The authors would like to acknowledge prof Józef Dulak and Janusz Drebot of the UJ Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland for supplying the mice tails used in this study. The authors would also like to acknowledge prof. Piotr Suder for providing access to the tissue homogenizer used in this study.

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MECHANICAL PROPERTIES OF POLYMER BEADS AFTER IMMERSION IN SOLUTIONS OF DIFFERENT pH

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[ENGINEERING OF BIOMATERIALS 163 (2021) 110]

Introduction

Natural polymers, such as sodium alginate and gellan gum, are widely used in skin care products, medicine, and materials science. These polysaccharides are biodegradable, non-toxic, and biocompatible [1]. Gellan gum and sodium alginate are used for the production of transporting active substance particles and as a platform for drug delivery systems [2].

Sodium alginate is an anionic linear polysaccharide extracted mainly from brown algae [3]. It is composed of α-L-guluronic acid and β-D-mannuronic acid residues linked by a glycosidic bond [4]. Sodium alginate can transform into a hydrogel by reacting with divalent cations such as Ca^{2+} and Ba^{2+} [5].

Gellan gum is another anionic polysaccharide, similar in utility profile to alginate. Gellan gum forms transparent heat-resistant gels [6]. It consists of repeating units of d-glucose, l-rhamnose, and d-glucuronic acid [7].

Decyl glucoside (DG), as a long-chain alkyl glucoside (FIG. 1), has been applied in cosmetic formulations as a mild non-ionic surfactant with excellent washing properties, biodegradability and low toxicity [8].

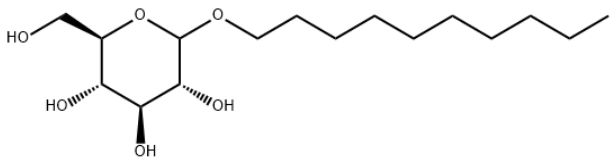

FIG. 1. Structure of decyl glucoside.

The aim of this study was to produce beads based on biodegradable polymers (sodium alginate and gellan gum) containing surfactant (decyl-glucoside) to provide the washing properties of the beads. The mechanical properties of the beads after immersion in solutions with acidic, neutral, and alkaline pH were examined.

Materials and Methods

Sodium alginate (ALG) was supplied by BÜCHI Labortechnik AG (Flawil, Switzerland). Gellan gum (GG) was purchased from Sigma-Aldrich (Poznan, Poland). Calcium chloride was supplied by Stanlab (Lublin, Poland). Decyl glucoside (DG) was acquired from Greenaction (Kielce, Poland).

Beads based on sodium alginate (ALG) and gellan gum (GG) containing non-ionic surfactant (Decyl Glucoside) were prepared by extrusion method. Polymer solutions with surfactant were put into a syringe with a needle (diameter 1.2 mm) and dropped to crosslinking solution (0.5M $CaCl_2$).

The obtained beads were immersed in solutions:

- Sodium acetate buffer (pH = 4) – acidic
- Phosphate saline buffer (pH = 7) – neutral
- 1% Na_2HCO_3 (pH = 9) – alkaline

The mechanical properties of the polymer beads were conducted at room temperature using a mechanical testing machine equipped with compression jigs (EZ-Test SX Texture Analyzer, Shimadzu, Kyoto, Japan). The wet samples and after 2 h of immersion in different conditions were examined. The tests were carried out at a compression speed of 1 mm/min up to 50% of strain. Young's modulus was determined. The results were recorded by Trapezium X software.

Results and Discussion

The obtained results showed that gellan gum beads exhibited higher values of Young's modulus compared to sodium alginate beads. The GG samples were stiffer, while the ALG samples were more flexible.

The values of elastic moduli were also dependent on the pH of immersing solution. In the case of both types of samples, the values of Young's modulus increase with the higher pH of solutions. The beads were stiffer in alkaline solution than at acidic pH.

Conclusions

The beads' stability in different pH is essential for the application to the skin and used in skin care products and drugs. Moreover, materials with cleaning agents should indicate appropriate pH to effectively remove dirt and other contaminants.

The next stage of the research will be the introduction of beads based on natural polymers with a surfactant into the matrices which can be used as skin-safe and biodegradable cleaning products.

Acknowledgments

Financial support from the National Science Centre (NCN, Krakow, Poland) Grant No 2018/31/N/ST8/02007 is gratefully acknowledged.

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INVESTIGATION ON THE DEGRADATION RATE OF PCL POLYMER SCAFFOLDS WITH BIOGLASS AND GRAPHENE ADDITIVES USING COMPUTER MICROTOMOGRAPHY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 111]

Introduction

Scientific research describing the phenomenon of degradation of bioresorbable polymers intended for medical applications such as PLLA, PGLA or PCL is carried out on the basis of the ISO 10993 standard for medical devices. These tests are based on the measurement of both changes in the material (mainly related to the surface characteristics or mass change) and the medium in which the material is incubated. Depending on the material tested, surface changes used by optical microscopy or SEM can be observed after several months of incubation. Due to the resolution obtained with the use of non-destructive computer microtomography technique (up to 0.5μmm), volumetric changes of the tested samples can be observed earlier, which makes it possible to better understand the phenomena related to the degradation of the material.

Materials and Methods

The research material consisted of four groups of scaffolds: PCL, PCL with bioglass, PCL with bioglass and zinc, and PCL with graphene produced by 3D printing, FDM technique [1,2]. Original Prusa® i3 MK3 printer, working in the FDM technique, was used for printing. The bed temperature to increase the adhesion for the first layer was 60°C and the extruder temperature was 190°C. The duration of the printout was about 14-15 minutes. The base material was pure poly (ε-caprolactone) PCL, (Sigma Aldrich) with a molecular weight of 80 kDa. Measurement samples with dimensions of 10x19.7x3 mm were prepared from three layers that are arranged alternately at an angle of 90°. The height and width of each layer are 1 mm and the distance between the tracks is 0.7 mm. For each of the studied groups, 5 measurement samples were prepared. Samples were incubated in PBS solution. Measuring the weight of scaffolds and the pH value, conductivity and concentration of Na^+, K^+, Ca^{2+} ions in the incubation fluid, as well as qualitative and quantitative measurement of structural properties using computer microtomography assessed the *in vitro* degradation of materials.
The registration of samples with a resolution of 10μm and lamp parameters 40kV/250μA, was performed using a SkyScan 1172 computer microtomograph by Bruker®.
A series of tests was carried out over a period of 22 weeks, the first stage was 14 days later and each subsequent stage was 30 days apart, according to standard 10993-13.

Results and Discussion

Research with the use of microtomography and dedicated programs such as DataViewer and CtAn (Bruker) allowed for the qualitative and quantitative assessment of changes occurring at individual stages of polymer degradation. The possibility of comparing and submitting several reconstructions of the same samples obtained in different stages of degradation allowed to obtain pictures of the differences of the samples before and after 22 weeks of incubation, which was 6 months. These effects for exemplary PCL samples with bioglass and graphene are shown in FIG. 1.

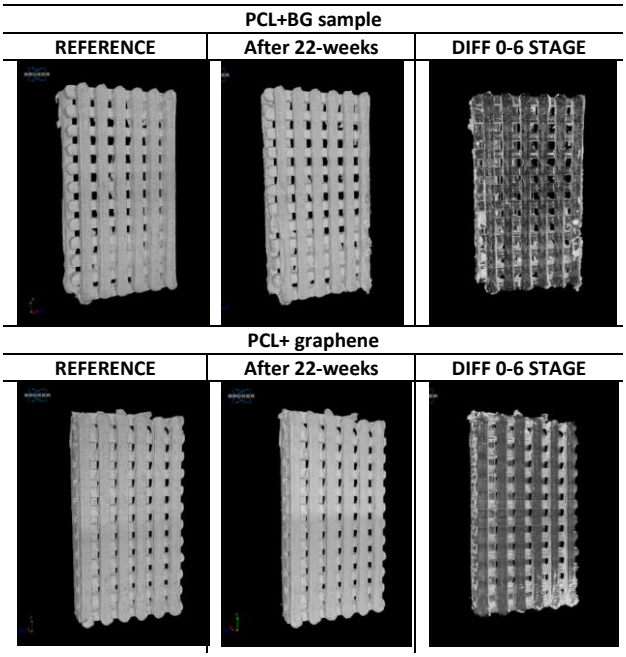

FIG. 1. Comparison of the reconstruction of sample samples: PCL with bioglass and PCL with graphene, along with a representation of the differences of these samples before and after 6 months of incubation

Conclusions

In all the scaffolds tested, a similar pattern of degradation can be seen. The comparative analysis of the individual stages allowed the observation that the corners of the samples change first, only then the center. A characteristic "cross" is formed. On the other hand, quantitative studies showed the greatest changes in the tested parameters (pH, mass and conductivity) between the 4th and 5th stage of incubation. The structural changes were associated with a decrease in the concentration of Na^+, K^+ and Ca^{2+} ions in the fifth stage of degradation. The research presented here covers a period of 6 months, but it is only a fragment of the designed experiment, which is assumed to last 18 months, thanks to which it will be possible to obtain information on a longer degradation time.

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COMPARATIVE STUDIES OF SELECTED PHYSICAL AND MECHANICAL PROPERTIES OF TOOTH TISSUES FROM PRIMARY TEETH

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[ENGINEERING OF BIOMATERIALS 163 (2021) 112]

Introduction

Damaged tooth tissues cause pain, which means that they are unable to fulfill their basic functions. Once damaged tissue never returns to its original state, therefore, in order to prevent further disease progression, the damaged tissue should be removed and reconstructed using dedicated materials. Dental tissue diseases more and more often concern children for whom it becomes necessary to introduce dental restorations in primary teeth.

The main task of the dental filling is to rebuild the continuity of the tissue and take over its functions. The selection of the appropriate material requires a good knowledge of the mechanical and physical properties of the tooth tissues. These properties, however, depend on many factors, such as: the type of teeth (permanent teeth and primary teeth), the patient's age, the content of minerals and genes.

The selected material should replace the tooth tissue as well as possible in terms of mechanical properties, a filling that is too weak may be damaged, it may fall out or crumble, while a filling with too high mechanical properties may destroy healthy surrounding tissues.

Materials and Methods

The research materials were human primary teeth (N=12), consisting of: 1 pre-tooth (fetal tooth), teeth marked according to the Viohl classification (ISO-3950) as 63, 64 and 65 from children aged 2-6 years. The study used 2 preparations with caries (65_P), and 2 with Nano_Comfort filling. The Nano Comfort filling is composite hybrid filler, which is characterized by reduced polymerization shrinkage [1]. In order to determine the structural and physical properties, the scope of the research was divided into several stages. The first step was to register each of samples with a resolution of 9µm and lamp parameters 80kV/124µA, was performed using a SkyScan 1172 computer microtomograph by Bruker®.

This study allowed for the visualization of individual tooth tissues and filling, which in this case allowed to assess the quality of the connection between the tooth tissues and the filling. The next stage of the work was the measurement of the mineral density of dental tissues, carried out with the use of CTAn software and phantoms of a specific density. The last stage of the research was the measurement of Young's modulus (Olivier-Pharr method) and microhardness using the CSM MicroCombi Tester™ microhardness tester. On each of the teeth, measurement points were selected on the enamel and dentine, and on samples with filling, also points on the filling. The samples were loaded with the selected force of 300 mN with a break of 15 seconds after reaching the maximum value of the force.

Results and Discussion

Based on obtained results, it can be seen that the values of hardness and Young's modulus are the highest for enamel, the values for dentin are approximately 3-5 times lower (FIG. 1), which is also consistent with the works of other authors [2]. The Young's modulus values for enamel ranged from 49 to 98.8 GPa, which gave a difference of about 50%, similarly in the case of microhardness, the values ranging from 270 to 569 HV. For dentin, these values ranged from 9.78 to 64.14 GPa, the results differed by a maximum of about 85%, and 54 to 282 HV, which is a difference of even 81%. The results obtained for the dentine of primary teeth are also lower than those obtained for enamel, for the Young's modulus by an average of 56%, for hardness of 65%.

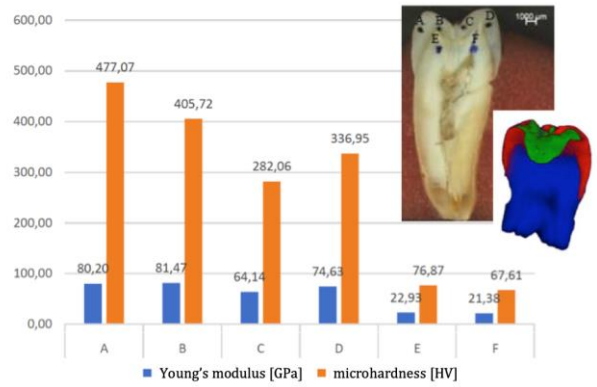

FIG. 1. Comparison of the Young's modulus and microhardness values for different regions of the exemplary tooth 85.

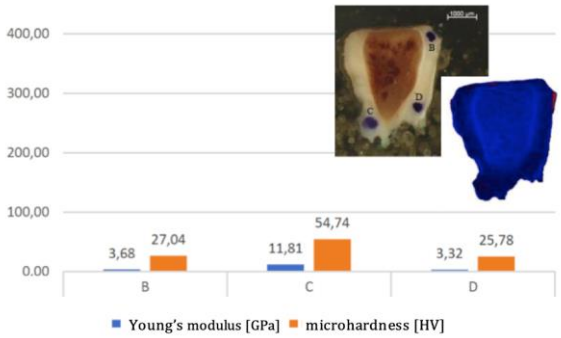

FIG. 2. Comparison of the Young's modulus and microhardness values for different regions of fetal tooth.

A very interesting case is also the fetal tooth, one of the alleged causes of which may be a deficiency of minerals in the mother during pregnancy (FIG. 2). This tooth is characterized by the lowest values of both Young's modulus (3-11 GPa) and microhardness (25-54 HV).

Conclusions

The results for all examined tissues differed not only between the examined teeth, but even within the same tooth there were high differences between the results. The influence of the type of teeth (1-5), the age of patients and the condition of the teeth on the tested properties was also observed. The more developed and mature the tooth, the higher the mechanical properties - this is due to the fact that the incisors are responsible only for biting food, and the teeth located in the back part of the mouth are responsible for the mechanical processing of food. The jaw forces acting on the teeth when grinding food are greater than when biting bites.

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INFLUENCE OF DLC COATINGS MODIFICATION ON LOAD TRANSFER BY THE GUIDED GROWTH STABILIZATION SYSTEM MODELLING THE SPINE DURING THE AGE OF GROWTH

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[ENGINEERING OF BIOMATERIALS 163 (2021) 113]

Introduction

The current internal stabilization systems of the spine, related to the treatment of scoliosis in children, enable the elimination of the need for multiple operations. The use of kinetic pairs (screw-rod) in the stabilization system makes their relative displacement during the child's growth is possible. The friction between the sliding elements made of titanium alloys contributes to the wear of the material, the particles of which are deposited, among others, in the tissue surrounding the stabilizer and form the so-called “tissue tattoo” [1]. In our research, to eliminate frictional wear, an applied DLC (Diamond-Like Carbon) coatings in kinematic pairs, which, as the research shows, is used as coatings for implants [3]. This material is characterized by high hardness and abrasion resistance, and at the same time, it is biocompatible with tissues.
Our research aimed to evaluate the stabilization components' mechanical properties and friction wear without modification (made of a titanium alloy) and after modification with Diamond-Like Carbon coatings.

Materials and Methods

The research was carried out on spine preparations (Th11-L7) collected from pigs aged 8-10 months and weighing 80÷90 kg. The stabilization system used to follow the growing spine in children SOCORE GGS by NovaSpine consisted of a four-segment stabilization with four central polyaxial screws permanently fixed and with “loose” fixing of the extreme screws (use of movable nuts). The tests also included a stabilization system in which the surface of the rod and the sliding nuts made of Ti-6Al-4V alloy was applied a 1000 nm thick DLC coatings (with the PVD method with CrN interlayer).
To analyse the cooperation of the spine- stabilization system, a long-term cyclic load test using a MTS Bionix® 858 was carried out for 100,000 cycles with a frequency of 1.5 Hz [2]. To assess the wear of the titanium surfaces of the stabilizer elements, tests were carried out on the nuts before and after the operation. The research was carried out using scanning electron microscopy (Jeol JSM 6610A) and an optical profilometer (Leica Filmetrics).

Results and Discussion

Based on the obtained data, the force-displacement characteristics of the tested specimens were determined, which determined the value of the height loss (H) and the stiffness coefficient.
The dynamics of changes in the stiffness coefficient value for the studied groups were similar and characterized by an initial slow increase, lasting up to 80,000 cycles. In subsequent cycles, there was no visible further increase in the stiffness coefficient, and its value in subsequent cycles remained at a similar level.
The modification of the stabilization with the DLC coatings decreased the stiffness by about 6% to the stabilization without the coatings. The *k* value for 100,000 cycles for the spine without the stabilization was 2778 N/mm and was 15% lower than the stabilization without the coatings and 10% lower than the stabilization after modification with the DLC coatings.
The value of the height loss for 100,000 cycles was 8.76±0.62 mm for the stabilization without the coatings, while after modification with the DLC coatings, it was 9.32±1.67 mm.

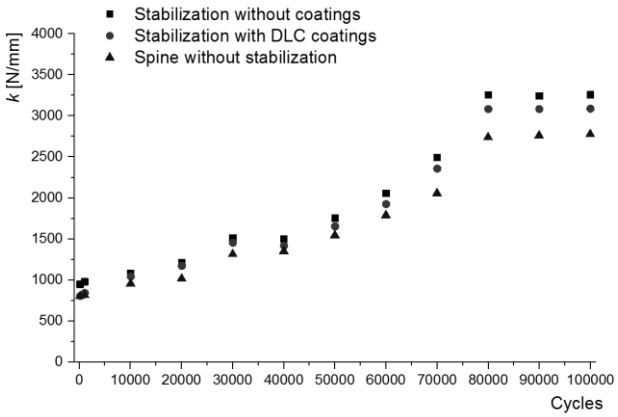

FIG. 1. The average value of the stiffness coefficient (*k*) in subsequent cycles for the studied groups.

The surface friction wear of the nuts was greater in the stabilization without coatings modification than after modification with the DLC coatings. More intense scratches and characteristic abrasions were observed in the places of cooperation between the rod and the movable nut. This results from greater friction of the nuts along the rod (where formed the kinematic knot responsible for the passive "following" effect). This means that the surface of the nuts made of titanium alloys without the DLC coatings was exposed to higher tribological loads during the stabilizer operation.

Conclusions

Based on the conducted long-term cyclic load test, it can conclude that the use of the DLC coatings in the guided growth stabilization system modelling the spine during the age of growth resulted in:
- decreasing the stiffness of the stabilization system,
- increasing the abrasion resistance in kinematic pairs.

Acknowledgments

Work to be implemented under the Intelligent Development Operational Program 2014-2020 - Project no. POIR.04.01.01-00-0020/19-00.

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MECHANICAL PROPERTIES OF TITANIUM ALLOY MESHES USED IN INTERBODY FUSION CAGE

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[ENGINEERING OF BIOMATERIALS 163 (2021) 114]

Introduction

Interbody fusion cages fill the interbody space while ensuring a stable connection at the implant-bone interface. Cages made of titanium alloy (Ti6Al4V) are commonly used in spine surgery due to their mechanical strength and much better osseointegration than PEEK cages [2,3]. Early bone fusion is favoured by adjusting the stiffness and eliminating the stress shielding effect, mainly by introducing to the cage surface porosity or scaffolding in the shape of mesh structures.
In the technology of producing implants, 3D printing is prevalent, allowing the suspension of the contact surface between the material and the bone by adjusting the design and filling density. In this way, it is possible to produce implants with a relatively small filling, characterized by favourable mechanical properties favouring cell adhesion and osseointegration of the implant-bone tissue [1,4].
The research aimed to determine the mechanical parameters based on the indentation test of titanium alloy meshes used in interbody fusion cage.

Materials and Methods

The research was carried out on meshes obtained by 3D printing, the structure of which was based on the connection of two six-armed pyramids described by the dimensions: width of the shoulder spacing (W), the height of the arms connection between pyramids (h), the height of the elements corresponding to the distance between the two vertices of the pyramids (H) - TABLE 1.
Meshes were made of titanium alloy Ti6Al4V (ELI) powder with a particle diameter of ± 50 μm. The printing was carried out on an EOS M280 printer with a laser beam diameter of 90 μm and a power of 200W. To improve the mechanical properties, printed titanium meshes were treated at 800°C (1470°F) for 4 hours in argon inert atmosphere.
The indentation test (spherical indenter with a diameter of 5 mm) was carried out at a speed of 2 mm/min to damage the mesh using the MTS 858 Mini Bionix testing machine. Then, performed a microscopic analysis of the damage resulting from the indentation test using a light microscope.

TABLE 1. Geometric dimensions of the tested meshes.

Samples	Geometric dimensions		
	W	h	H
	[mm]		
S3	0,20	1,8	1,8
S4	0,24	1,8	1,8
S5	0,20	2,0	1,8
S6	0,24	2,0	1,8
S7	0,20	1,8	1,6
S8	0,24	1,8	1,6
S9	0,20	2,0	1,6
S10	0,24	2,0	1,6

Results and Discussion

The mechanical parameters for each group of meshes were determined based on the indentation studies, as shown in FIG. 1. The analysis of the maximum force value and the stiffness coefficient showed that higher values are found in the groups in which the height of the arms connection between pyramids (h) is 0.24 mm than in the groups in which this value was 0.20 mm.
The highest values were found in the S8 group, for which the value of the maximum force was 473±32 N and the value of the stiffness coefficient was 231±6 N/mm.
On the other hand, the lowest values were found in group S9 where for which the value of the maximum force was 276±16 N the value of the stiffness coefficient was 61±3 N.

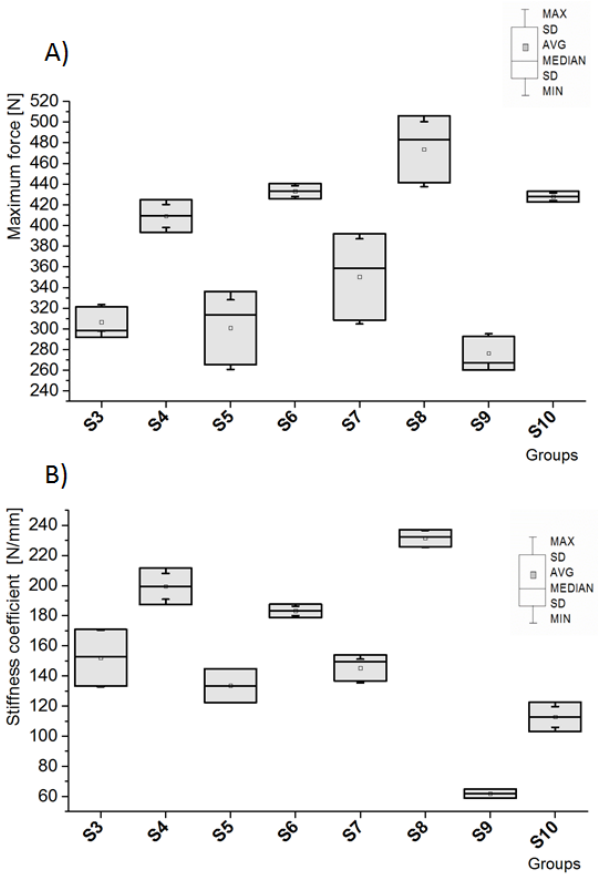

FIG. 1. The average value of a) the maximum force; b) the stiffness coefficient (k) of the tested groups of meshes.

Conclusions

The most significant impact showed the dimension of the height of the arms connection between pyramids (h). After the indentation test, the microscopic analysis of the meshes showed no discrepancies in the mechanism of damage arising from geometric differences.

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INFLUENCE OF HEAT TREATMENT OF CARBON NANOFIBERS ON GENOTOXICITY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 115]

Introduction

The problem of the toxicity of nanomaterials is an open question. There are reports on the positive effect of various nanoparticles, including carbon nanoparticles, on the cellular and tissue response, as well as the results indicating their toxic or genotoxic effects, especially if their release and transport in the body is uncontrolled [1,2]. This problem concerns both nanoparticles that are introduced into the body as drug carriers or components of implants for tissue regeneration, as well as the problem with their release into the environment, for example into water reservoirs from various industries, and further negative impact on aquatic organisms, animals and humans. Our previous research indicates that the degradation products of electrospun carbon nanofibers (eCNFs) are not genotoxic compared to unmodified carbon nanotubes (CNTs), which exhibit some degree of genotoxicity [3,4]. However, CNFs can be obtained at different temperatures, and as it is known, this factor affects the degree of crystallinity of these nanomaterials, which may have a direct impact on the cytotoxicity and genotoxicity of their degradation products. Therefore, the main aim of the study is to evaluate the influence of ground CNFs obtained at different temperatures on genotoxicity.

Materials and Methods

The conversion of polyacrylonitrile (PAN) nanofibers obtained using electrospinning method into carbon nanofibers (CNFs) consisted of two steps i.e. stabilization and carbonization. The stabilization process was carried out by heating the nanofibers at a rate of 3°C/min to 250°C, keeping them at this temperature for 30 minutes, then additional heating to 270°C and keeping them for another 20 min. The carbonization process was carried out in a quartz and graphite tube furnaces by heating of stabilized nanofibers in nitrogen atmosphere at several temperatures i.e. 750ºC, 1000ºC, 1500ºC, 1750ºC and 2000°C at a heating rate of 7°C/min, without holding the samples at final temperature. The samples were named as follows: CNF_750, CNF_1000, CNF_1500, CNF_1750 and CNF_2000. All samples were investigated using different methods like SEM for morphology and microstructure investigation, Raman spectroscopy and XRD for structural investigation and goniometer for analysing of surface wettability. Chondrocytes (CHON-001 cell line) were contacted with CNF at 37°C for 24 h; next, the cells were washed in PBS and analysed by comet assay procedure.

Results and Discussion

Structural studies carried out by means of Raman spectroscopy and X-ray diffraction confirm the influence of temperature on the degree of crystallinity of the obtained CNF. As expected, the highest crystallinity was observed for the CNF_2000 sample which was carbonized at the highest temperature. Also the value of the parameter d002 decreases with increasing temperatures, which confirms the influence of temperature on the structure ordering of CNF.
The carbonization temperature has also impact on surface wettability of CNFs. The highest contact value was observed for CNF_2000 while the lowest for CNF_750 (TABLE 1). The decrease of wettability with the CNFs treatment temperature is related to the increase in crystallinity as well as the removal of functional groups, especially oxygen ones, from the surface of nanofibers.

TABLE 1. Water contact angle for CNFs after carbonization process in different temperatures.

Samples	Water contact angle [º]
CNF_750	97,83±17,95
CNF_1000	122,37±3,04
CNF_1500	122,38±2,77
CNF_1750	125,70±4,57
CNF_2000	134,32±1,75

Genotoxicity of the CNFs has been assessed using the analysis of comets, in terms of the tail DNA, as shown in FIG. 1. Genotoxicity testing was performed after 24h incubation for two concentrations of ground CNFs. These results show a significant increase in genotoxicity for the CNF_750 and CNF_1000 samples at the higher concentration compared to the control. No increase in genotoxicity was observed for the remaining samples. On the other hand, no significant increase in cell death was observed for all tested nanofibers.

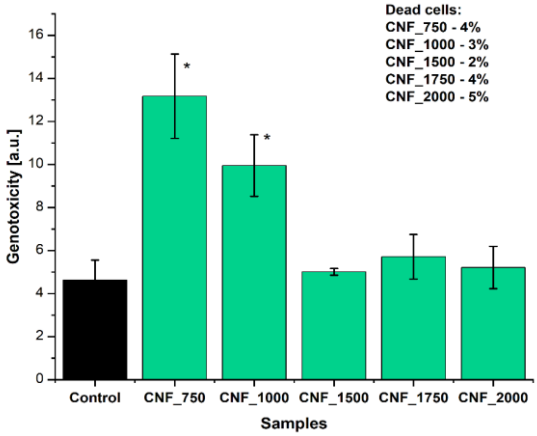

FIG. 1. Genotoxicity of CNF in chondrocyte cell line estimated by use t-DNA comet assay parameter for time of chemical incubation τ. Statistical analysis was performed using t-Student test. *p<0.05 vs. control.

Conclusions

These results indicate an increase in the degree of crystallinity of CNFs with increasing carbonization temperature. However, as indicated by the results of biological tests, it is not the degree of ordering structure and the increase of CNF crystallinity affects its genotoxicity, but most likely the presence of the amorphous phase and the remaining groups derived from PAN, in particular nitrile groups.

Acknowledgments

Research project supported by program “Excellence initiative – research university” for the AGH University of Science and Technology”. Project ID 1580.

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NEWLY CROSSLINKED CHITOSAN-BASED HYDROGEL BIOMATERIALS: PHYSICAL, CHEMICAL AND MECHANICAL INVESTIGATION

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[Engineering of Biomaterials 163 (2021) 116]

Introduction

Hydrogel materials are well known for their ability to absorb large amounts of water while maintaining the structure due to the crosslinking of polymer chains. They have attracted a great attention for their potential application in a lot of biomedical areas such as tissue engineering, dressing materials and controlled drug delivery systems. Hydrogels have a number of advantageous properties - the ability to mimic biomechanical characteristics of native extracellular matrix (ECM), ensuring an appropriate microenvironment for cells and promoting the transport of nutrients. Furthermore, their porosity, high swelling ability, and hydrophilic nature make hydrogels excellent candidates as carriers of hydrophilic biologically active compounds. Generally, all of these properties of hydrogels are highly associated with the degree of crosslinking.

Materials and Methods

In this study, porous hydrogel materials were produced based on two biopolymers - chitosan (CS) and pectin (PC), crosslinked with phenolic monoaldehyde - 2,3,4-trihydroxybenzaldehyde (THBA). Additionally, a polyphenolic compound - rosmarinic acid (RA) and a calcium-rich sol-gel-derived bioactive glass (BG) were used as functional components. The aim of the research was to assess the impact of the presence of individual substrates on the biopolymer cross-linking process as well as the final properties of the obtained hydrogels, including the microstructure (SEM/EDX, μ-CT) mechanical and thermal analysis (TG). Moreover, hydrogels were analyzed using ATR-FTIR spectroscopy.

Results and Discussion

μCT analysis of crosslinked hydrogels proved nearly 100% interconnectivity of the pores and high porosity (94.9% – 96.5%), regardless of the composition of the hydrogels. The crosslinking with THBA resulted in improved mechanical properties of materials (FIG. 1). Formation of the Schiff base in the chitosan matrix was confirmed by development of a distinct yellow colour. The FTIR spectra of crosslinked hydrogels showed an absorption band at 1628 cm^{-1}, corresponding to the stretching vibration of imine bonding. Furthermore, an absorption band of the phenolic hydroxyl groups of THBA shifted from 1279 to 1268 cm^{-1}, which may be due to the H-bonding between CS and THBA. When analysing the TG curves, crosslinked materials showed lower water content as well as enhanced thermal stability compared to uncrosslinked hydrogels, confirming the presence of covalent Schiff base bonding. Moreover, in the case of uncrosslinked materials, temperature of thermal decomposition of CS-PC hydrogels tended to be higher compared to CS materials, which may indicate ionic interactions between both polymers.

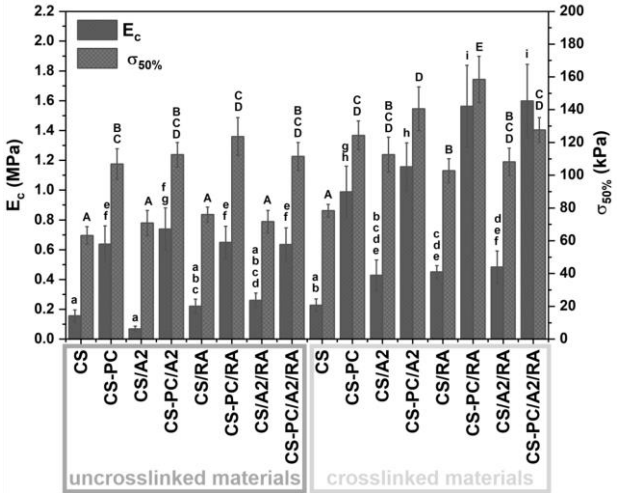

FIG. 1. Compression test results: Young's modulus and stresses corresponding to compression of a sample by 50% of the uncrosslinked and crosslinked hydrogels.

Conclusions

The presence of pectin, bioactive glass and rosmarinic acid, separately and in combination, affected the crosslinking process, while simultaneously modulating properties or imparting completely new ones. The obtained hydrogels represent promising multifunctional biomaterials with a wide range of physicochemical and mechanical properties with great potential for use in tissue engineering.

Acknowledgments

This work was supported by the National Science Centre, Poland, grant nos. 2017/27/B/ST8/00195 (KCK) and 2019/32/C/ST5/00386 (MD). This work was partly supported by program „Excellence initiative – research university" for the AGH University of Science and Technology.

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PHOTOSENSITIVE HEPARINS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 117]

Introduction

Heparin is a polysaccharide known for its important role in human physiology, mostly for its influence on blood coagulation and interaction with heparin-binding proteins, e.g., growth factors (FGF, VEGF) [1, 2]. In its native form heparin, like other polysaccharides, does not absorb light from the near ultraviolet/visible range of the spectrum, thus its biological activity is not responsive to light. Being a polysaccharide, heparin can be easily functionalized. Polysaccharides undergo facile esterification, etherification, and oxidation reactions [3]. Polysaccharides containing cis-trans photoisomerizable chromophores were also obtained. It was found that photoisomerization of these chromophores significantly changed the properties of the polysaccharides [4].
Therefore, it is postulated that functionalization of heparin with a photoisomerizable compound such as the arylazopyrazole (AAP)-type photoswitch, either by substitution or grafting, will allow gaining photocontrol over physicochemical properties of heparin (e.g., hydrophilicity/hydrophobicity, viscosity, solubility, chain conformation, interactions with other (macro)molecules), and, consequently, its biological activity.

Materials and Methods

Synthesis and characterization of photoactive UFH heparin

Since unfractionated heparin is scarcely soluble in organic solvents, it (Heparinum WZF, Polfa) was first converted into the ammonium salt soluble in DMF by reacting it with Hyamine 1622, using the method described in the literature [5]. In the heparin hyamate obtained the carboxyl groups are neutralized with sodium cations and the sulfate groups are neutralized with hyamine ammonium cations.
Then, the obtained salt was passed through a carboxyl ion-exchange Bio-rex 70 resin (in the acidic form) to obtain heparin form in which the sulfate groups were salified with hyamine 1622 and the carboxyl groups were in the reactive acidic form. The photoactive heparin derivatives were obtained via esterification of the carboxyl groups with AAP-type photoswitch. The esterification was performed using a method described in the literature [6]. For quantitative photoisomerization of the photoswitch attached to heparin 365 nm (trans-cis) and 530 nm (cis-trans) light was used. The half-life time of the cis isomer formed was checked by UV-Vis measurements. The influence of trans-cis isomerization on the hydrodynamic diameter was investigated by DLS method.

Results and Discussion

The applied synthesis pathway allowed the attachment of a photoswitch to unfractionated heparin (UFH). Depending on the ratio of the molar reactants, different degrees of substitution could be obtained. The trans-cis isomerization under 365 nm irradiation (FIG. 1) is quick (about 1 minute under LED irradiation), while the reverse process (cis-trans) under 530 nm irradiation was much longer (about 1 hour). DLS investigations of obtained derivatives of heparins showed that the trans-cis photoisomerization reduces the hydrodynamic diameter and this effect was more pronounced for higher degrees of UFH substitution. Studies of the cis isomer stability at different temperatures showed that the thermal half-life of the cis isomer in the physiological temperature is exceptionally long, opening possible biomedical applications for the photosensitive heparin.

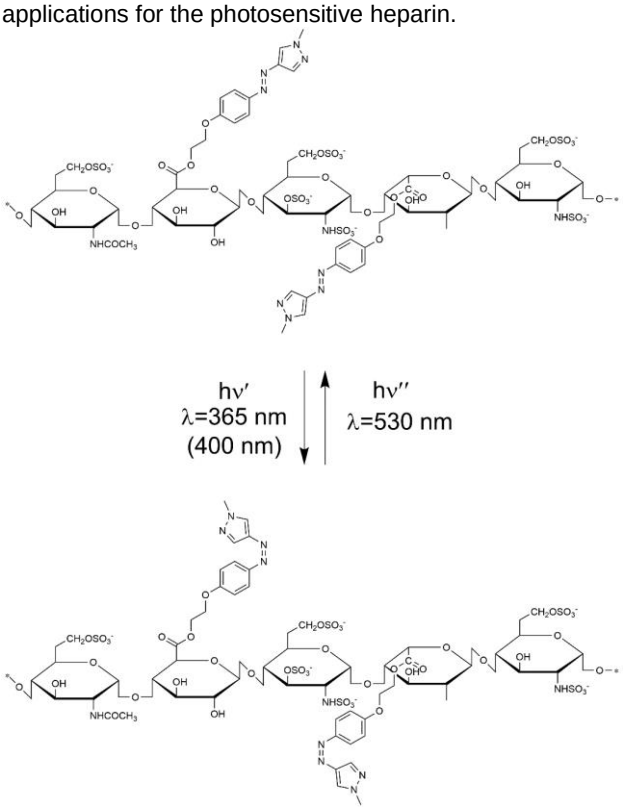

FIG. 1. Photoisomerizations in UFH heparin substituted with an AAP-type photoswitch.

Conclusions

Photosensitive heparins were obtained. Trans-cis and cis-trans isomerizations occur under 365 nm and 530 nm irradiation, respectively. The photoisomerization process influences the hydrodynamic diameter of UFH. Obtained photocontrollable systems are characterised by unusually long thermal half-life of the cis isomer.

Acknowledgments

Authors gratefully acknowledge the financial support from National Science Centre (grant UMO-2016/21/B/ ST5/008337).

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CONDUCTIVE POLYMER NANOCOMPOSITES FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 163 (2021) 118]

Introduction

Recently, polymer-based electro-conductive materials have been included in practical applications in the domain of medical therapy and diagnostics [1,2]. These materials occur to be indispensable for, among others, bio-sensor design, tissue engineering and regenerative medicine. Different high requirements and standards imposed by medicine very often are hard to be met by intrinsically conductive, conjugated polymers. In contrast, polymer nanocomposites can be much easier tailored to fulfil prerequisites for such applications. A particular place is occupied by carbon nanoforms. When introduced into a polymer matrix, they not only make it conductive, but can impart the desired mechanical properties and affect the surface parameters of the composite. Characteristics of this latter are decisive for the nature of interactions with the tissue and cell environment. To exploit this direction of research, we produced nanocomposites containing multiwall carbon nanotubes (MWCNT) or carbon nanofibres (CNF) dispersed in two different polysiloxane-based matrix. A part of samples contained functionalized derivatives of the above carbon nanoforms. Such a range of materials were thoroughly tested in terms of surface morphology, amount of carbon in the structure and electrical conductivity, and then compared and discussed.

Materials and Methods

MWCNTs were purchased form Nano-Amor, USA. Carbon nanofibers were obtained by fragmentation of ESCNF (electrospun carbon nanofibers) received by carbonization of polyacrylonitrile (PAN) precursor processed by electrospinning. Both types of carbon nanoforms were chemically treated in a mixture of acids (H_2SO_4, HNO_3), what ensured good dispersions in silkosan sol. The nanocomposites were produced using the sol-gel method. The siloxane sol containing dispersed carbon nanoforms was subjected to thermal treatment at 70°C for 7 days and then deposited on different solid substrates. The resulting nanocomposites were tested by mean of X-ray Photoelectron Spectroscopy (XPS), Energy Dispersive X-ray Spectroscopy (EDS), Scanning Electron Microscopy (SEM), contact angle (CA) technique and electrical conductivity (EC) as function of temperature.

Results and Discussion

Polymer nanocomposites containing MWCNT differ significantly in properties from composites with CNF. Both materials featured electrical conductivity, however higher in the case of composites with nanotubes. In samples containing CNF, a much stronger hopping character of the electrical conductivity was observed. CNF are longer and have a larger diameter than nanotubes. It is reasonable to assume that for this reason individual CNFs are more spatially separated. The resulting potential barriers are higher and more difficult to overcome for charge carriers, especially at cryogenic temperatures.

Analyzing the obtained XPS depth profiles, it was found that the carbon nanoforms were dispersed more homogeneously in the polysiloxane matrix containing phenyl groups. In the case of layers containing only methyl groups, there was observed a density gradient in the direction perpendicular to the sample surface.

Each of the nanoadditives has a different impact on the surface properties of the polymer. The topography of samples containing CNFs was much more developed comparing to the smooth and featureless surface of material containing MWCNTs. In contrast, the studied materials had hydrophilic surfaces regardless the type of the nanoadditive. differing only in the ratio of the energy components; dispersion and polarisation.

Conclusions

Polymer nanocomposites with carbon nanoforms have a high potential for medical applications. By choosing the content and the type of the nanoadditive, it is possible to vary significantly the material parameters, important for this domain. The results also proved that carbon nanotubes can be successfully replaced with carbon nanofibers. The biocompatibility of these latter has already been widely recognised, what opens the route to produce nanocomposites fully safe for the patients.

Acknowledgments

Authors acknowledge support by the National Science Centre, Poland under project “Polysiloxane layers modified by carbon nanotubes on metallic substrates” - 2017/25/B/ST8/02602.

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ANTIBACTERIAL PROPERTIES OF CARBON FIBERS MODIFIED WITH TITANIUM SOL

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[ENGINEERING OF BIOMATERIALS 163 (2021) 119]

Introduction

Thanks to the properties of the graphene layer containing π electrons, the surfaces of some carbon materials, both in the nano and micro scale, are materials with an extremely wide range of applications. These are applications related to carbon materials with antibacterial properties. It has been proven many times that nanofiber forms of carbon, such as CNT (carbon nanotubes), graphene, fullerene or CNF (carbon nanofibers) are materials with antibacterial properties [1-4].

However, their applications require the development of composite material systems in which carbon nanoforms are one of the constituents, i.e. modifier, giving the desired application properties [5,6].

On the contrary, micro-carbon fibers, especially made of organic precursors after thermal treatment at moderate temperature (low-carbonized carbon fibers) and subjected to intensive oxidation treatment, are a material not burdened with limitations related to the potential toxicity of carbon nanoforms, and additionally characterized by high functionality.

The aim of the study was to obtain carbon nonwovens made of fibers in a micrometric scale, and then to process them in titanium sol in order to give them antibacterial properties.

Materials and Methods

Carbon nonwovens were made of polyacrylonitrile nonwoven precursor, preliminary stabilized at oxidative atmosphere followed by carbonization at 1000°C and then additionally treated in an oxidizing medium. The material prepared in this way was surface modified with titanium sol. The subject of the study were the as-received carbon nonwovens and nonwovens modified with titanium sol. Both materials were characterized based on Raman spectroscopy, XPS research and SEM/EDS analysis. Then, the antibacterial activity of both materials was tested. The tests were carried out in accordance with the guidelines of the PN-EN ISO 20645 standard - Flat textiles - Determination of antibacterial activity - Diffusion method on an agar plate. The bacterial activity was analyzed against the reference bacterial strain: Escherichia coli ATCC 8739.

Results and Discussion

The results obtained in the study indicated that the carbon nonwovens being the subject of the study are materials with a low degree of crystallinity, with oxygen groups on their surface, characteristic for, the so-called "activated carbon" (carboxyl, carbonyl, lactone, phenol, bonded surface functional groups attached to the graphite-like layers' edges gives the acidic character of the activated carbon).

The spectroscopic data concerning the surface analysis of sol-modified nonwovens indicate that titanium dioxide with anatase structure is present on the surface of the fibers in the non-wovens. In the tests of antibacterial properties of both materials, carried out by the method of diffusion on agar plates, in which samples of the tested material were applied to the substrate inoculated with bacteria, the observation of the zone of inhibition of bacterial growth showed the antibacterial effect of fibers modified with titanium sol. Additionally, the test results were confirmed by the SEM observations (FIG. 1).

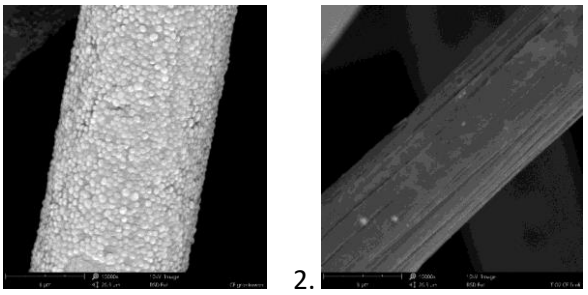

FIG. 1. SEM micrographs of carbon fibers after contact with bacteria (*Escherichia coli* ATCC 8739);
1- the surface of the unmodified fiber,
2- the fiber coated with titanium sol.

Conclusions

Modification of carbon nonwovens with oxygen groups on their surface with titanium sol is an effective method of manufacturing fibrous functional antibacterial materials that can be used in various types of filters intended for the purification of gaseous and liquid media.

Acknowledgments

This work has been supported by the National Science Centre, Poland under project "Polysiloxane layers modified by carbon nanotubes on metallic substrates" - 2017/25/B/ST8/02602

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GRADIENT SCAFFOLDS FOR THE REGENERATION OF OSTEOCHONDRAL DEFECTS OBTAINED USING 3D PRINTING TECHNOLOGY

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[ENGINEERING OF BIOMATERIALS 163 (2021) 120]

Introduction

Recovery of osteochondral defects is still a challenging task for surgeons across the world. Osteochondral tissue consists of bone, cartilage and bone-cartilage interface, and therefore is characterized by gradient of mechanical and biological properties. When designing scaffolds, it is important to properly design a porosity gradient. The porosity of the scaffold is influenced by the pore size, pore size distribution and geometry. The pore structure plays an essential role in cell adhesion, migration, proliferation and tissue formation as well as nutrient diffusion [1]. 3D printing technology is a very promising tool for osteochondral defects regeneration applications, as it allows for the creation of precisely designed, personalized implants.

The aim of the work was to design scaffolds with different porosity gradients based on the literature data. As the structural design plays a critical role in improving the mechanical properties of porous biomaterials. The second goal of this work was the selection of a scaffold that meets the requirements for porosity and mechanical properties.

Materials and Methods

Scaffolds were made of polycaprolactone (PCL, Sigma-Aldrich, Mw 80 kDa) using 3D printing in fused deposition modelling (FDM) technology. Samples were first designed using Autodesk Inventor and converted into FDM-printable files using Prusa Slicer software. Six different scaffolds 20 x 20 mm with different porosity gradient were designed and produced using Prusa i3 MK3 printer. SEM and µCT investigation of the scaffolds were performed in order to evaluate the porosity gradient along the printed scaffold. Scanning electron microscope (Nova NanoSEM 200, FEI, AGH) equipped with EDS analysis was used to evaluate scaffold microstructure. The samples were coated with carbon before observation. The architecture of the scaffolds was analyzed by micro-computed tomography (µCT) using a SkyScan 1172 Bruker® scanner.

Tensile tests have been performed using computerized universal testing machine (Zwick/Roell, Germany), according to EN ISO 527-2:1996. Samples with dimensions given in the standard were printed on a printer.

Results and Discussion

Size of the osteoblasts is in the range of 10-50 µm, however preferred scaffold pore size for osteoblasts proliferation is in the range of 100 – 200 µm [2].

6 scaffolds with different pore size, geometry and shape were designed. Among others, scaffold with a pore size of 100-200 µm in the chondral layer and a pore size of 300-450 µm in the osseous layer [3] were prepared.

An example of designed scaffold used in the research is shown in FIG. 1.

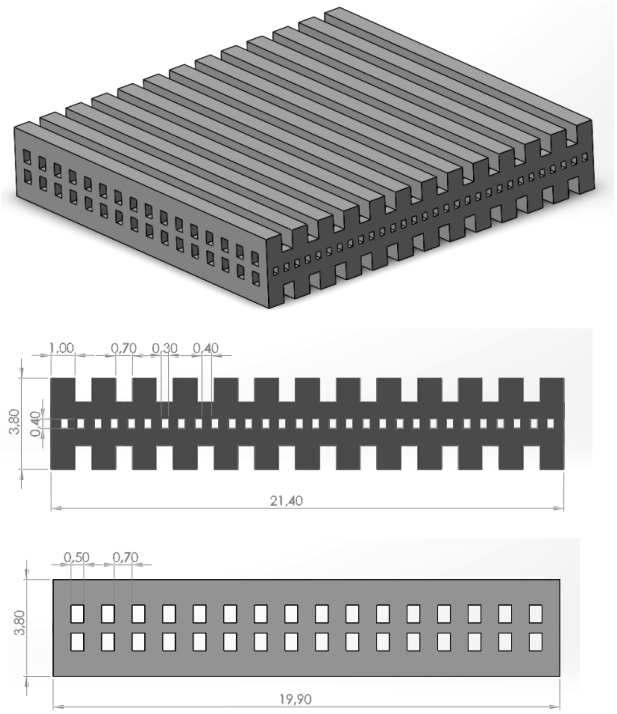

FIG. 2. Example of gradient scaffold structure used in the research.

All designed scaffold were printed and tested. The microscopic observation confirmed the presence of pores with different geometries along the scaffold. The scaffolds varied in mechanical properties.

Conclusions

Preparation of biocompatible gradient scaffold for osteochondral face defect treatment should facilitate implantation, increase recovery rate and improve the final appearance of the patient.

Further studies will focus on designing more advanced, perhaps multi-material gradient scaffolds and implementation of the 4D printing technology into implant preparation process.

Acknowledgments

This work was supported by the National Science Centre, Poland in the frame of project: “Layered scaffolds for nasal cartilages reconstruction fabricated by 3D printing and electrospinning” 2015/18/E/ST5/00189 (Sonata Bis 5) and by The National Centre for Research and Development in the frame of project: “Layered implants for nasal cartilages and bones reconstruction fabricated by 3D printing” TANGO-IV-A/0002/2019.

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