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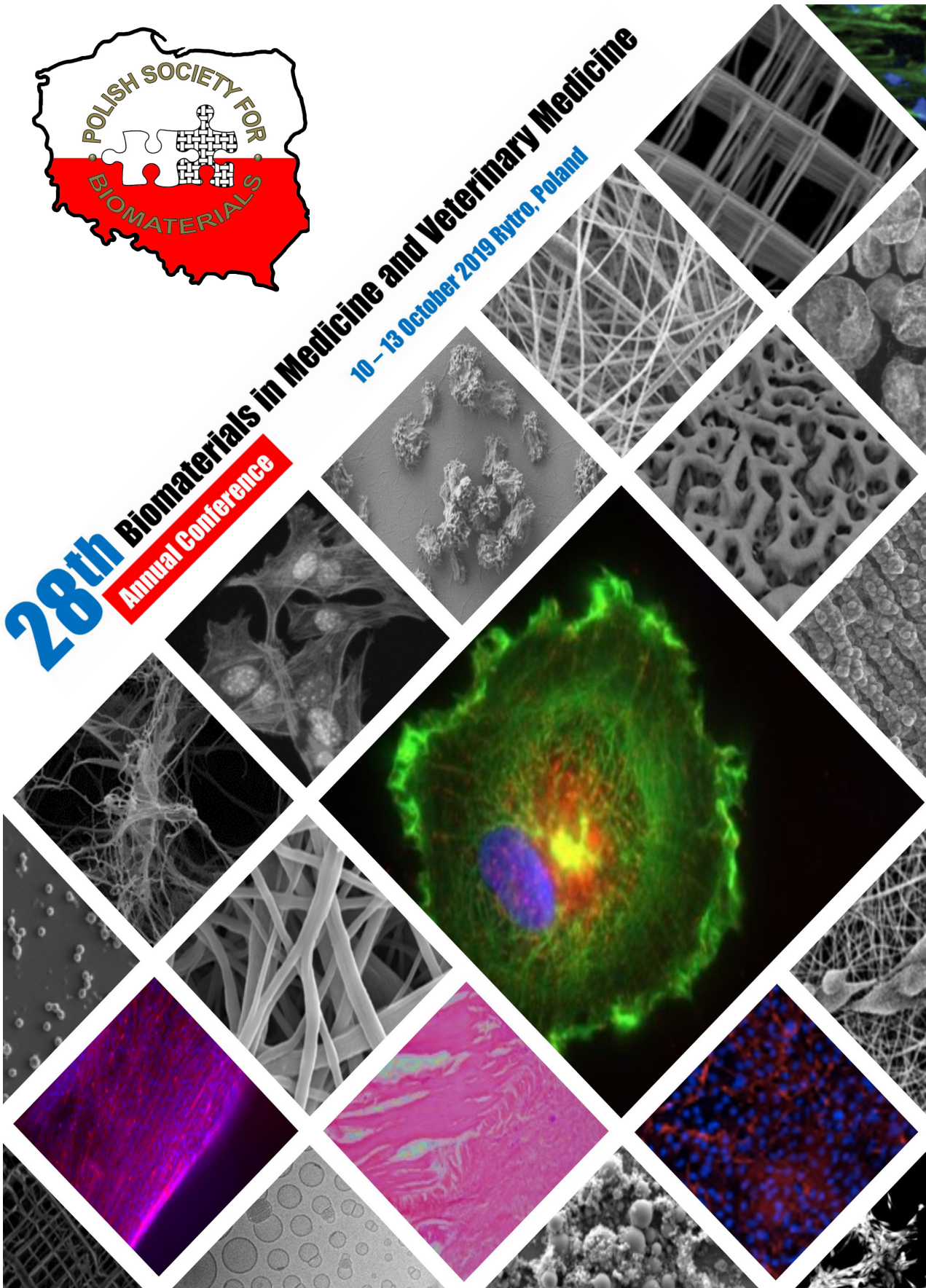

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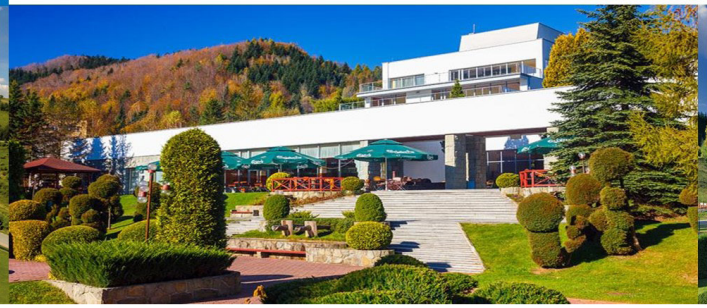

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3D BIOFABRICATION FOR TISSUE ENGINEERING

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Introduction

Typical tissue engineering approach is to fabricate porous scaffold, and then to seed it with patient own cells and growth factors, before implantation. Difficulties with cell seeding and providing a proper 3D ECM-like environment for the cells are the main limitations of this approach. An innovative technique that may overcome current limits in reproducing complex structures of human tissues and organs is 3D biofabrication. This emerging fabrication technology relies on the simultaneous deposition of cells and biomaterials, mostly in a layer-by-layer fashion, to form 3D well-organized living heterogeneous porous structures that can mirror physiologically and morphologically relevant complex biological architectures. The aim of the study was to biofabricate biomimetic 3D models for tissue engineering of musculoskeletal tissues like muscle, tendon, or cartilage.

Materials and Methods

Innovative strategies to biofabricate biomimetic 3D models of musculoskeletal tissues, like cartilage, muscle, and tendon are presented. The 3D biofabrication approach is based on a microfluidic system coupled to a co-axial needle extruder for high-resolution computer-controlled 3D deposition of hydrogel fibers laden with different type of cells (FIG. 1a). In the first step formulations of ECM mimicking tailored hydrogel based bioinks were developed. Depending on application, the biomimetic hydrogels were composed of modified biopolymers like gelatin, alginate, hyaluronic acid, or PEG-fibrinogen. The gels were laden with different types of cells including bone marrow-derived human mesenchymal stem cells, muscle precursor cells or chondrocytes. Then 3D bioprinter and bioinks were used to precisely reproduce a 3D spatial organization of natural musculoskeletal tissues. The 3D printed advanced biostructures were cultured in static or dynamic conditions to develop into neo-tissues of musculoskeletal system.

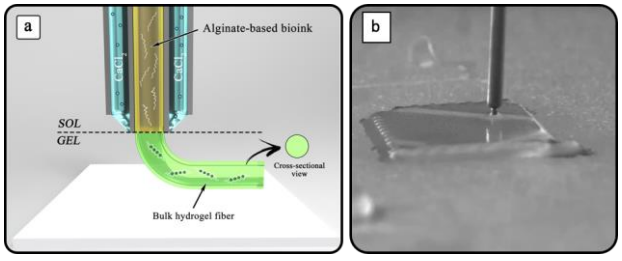

FIG. 1. Co-axial nozzle (a) for high-resolution 3D printing of hydrogel fibers (b).

Results and Discussion

By formulating tailored hydrogel based bioinks and precisely controlling the 3D spatial organization of the extruded hydrogel fibers, it was possible to biofabricate advanced engineered living constructs mimicking natural musculoskeletal tissues. The obtained with high resolution (~ 100 μm), a fiber-based 3D printed living constructs mimiced organized tissues like cartilage (FIG. 2a,b) [1], muscle (FIG. 2c,d) [2], and tendon (FIG. 2e) [3]. Furthermore, the mechanical loading and biochemical stimulation enhanced ECM deposition in 3D biofabricated constructs (FIG. 2f) [3].

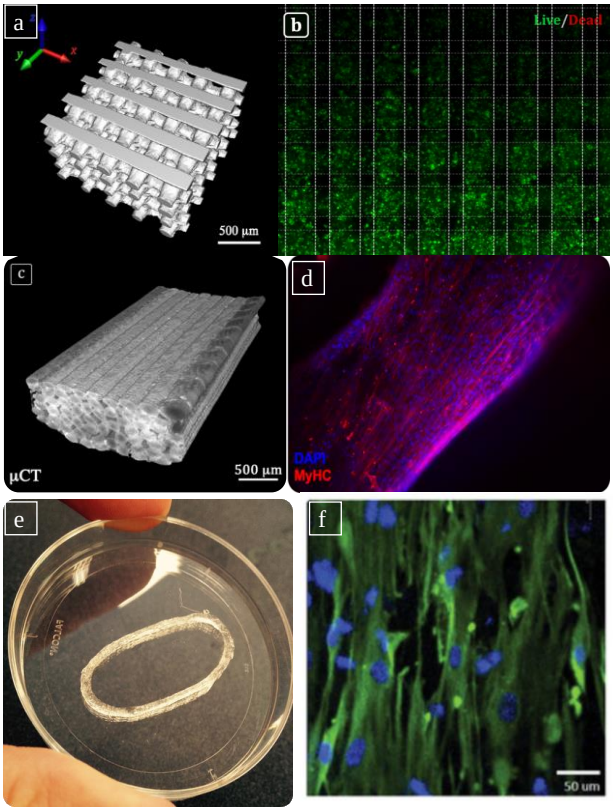

FIG. 2. 3D printed living constructs mimic organized tissues like cartilage (3D microCT image (a) and live-dead staining (b)) [1], muscle (3D microCT image (c) and immunofluorescence micrograph (d)) [2], and tendon (the ring of highly aligned, densely packed fibrous structures (e) and collagen I (green) expressed by hBM-MSCs encapsulated into the hydrogel yarns mechanically stimulated (f)) [3].

Conclusions

Properly designed bioinks and 3D biofabrication methods were crucial for development of 3D living constructs mimicking organized musculoskeletal tissues like cartilage, muscle, and tendon. Blending alginate with photocurable natural based polymers allowed to formulate ECM biomimetic inks that were used for high-resolution 3D microextrusion-based bioprinting a fiber-based 3D structures recapitulating architectures of native tissues. Additional post-processing biochemical and mechanical stimulation can induce MSC differentiation and enhance ECM deposition. In the next step, long-term in vivo evaluation of the biofabricated constructs are required before such tissue engineered products might be used in the medical practice.

Acknowledgments

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PROACTIVE BIOMATERIALS: BIOMIMETIC INTERFACIAL PROPERTIES AND ADVANCED DELIVERY SYSTEMS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 7]

Introduction

To heal and repair the human body, man has for centuries turned to biomaterials. Originally designed to be inert, contemporary applications such as tissue engineering and regenerative medicine demand biomaterials that can actively engage cellular matter, to direct and modulate the biological response, at the implant site and beyond. So, directing cell behavior is a key contemporary challenge in biomaterials science.
Since biomaterials interact with their surroundings via their surface, an attractive strategy toward truly bioactive materials is through the design of biofunctionalized coatings/interfaces. Of particular promise are three dimensional, cellularized, porous scaffold devices – with optimized interfaces to support and implant viable cells – promising breakthrough biomedical advances in the near future.

Results and Discussion

Engineering advanced materials, able to proactively and efficiently "dialogue" with surrounding tissues, is at the heart of the works developed at the University of Cergy-Pontoise, France in the Biomaterials for Health lab. Our activities are principally focused on the dynamics of extracellular matrix and biopolymer component assembly – at the molecular and supramolecular scales – in solution, at interfaces, and in biomaterials applications.
During the last decades, we made significant progress on projects that involves polyelectrolyte-based films, formed via layer-by-layer assembly.
In particular, strategies involving nanotemplating, to spatially and temporally release bioactive molecules (e.g. BMP2) [1], FIG. 1.

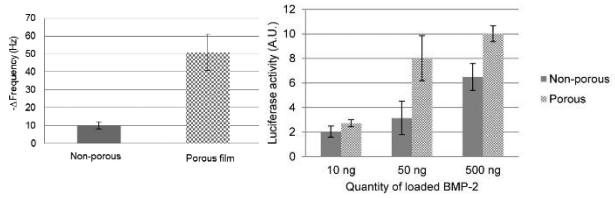

FIG. 1. Left, Quartz crystal microgravimetry measurements of BMP-2 loading onto/within cross-linked (PLL-PGA)14 (non-porous) and cross-linked ([PLL-PGA]5-PLL-NP)2-(PLL-PGA)2 exposed to THF (porous) films. Both films are incubated with 150 ng of BMP-2 (during 15 min) then extensively rinsed with buffer (10 min total). Right, Bioactivity induced by BMP-2 loaded onto/within non-porous and porous thin films. BMP-2 bioactivity is determined by measuring the luciferase expression of C2C12-BRE/Luc cells. The cells are cultured for 24 h on cross-linked (PLL-PGA)14 (non-porous) and cross-linked [(PLL-PGA)5- PLL-NP]2-(PLL-PGA)2 exposed to THF (porous) films loaded with 10, 50 or 500 ng/L of BMP-2. The results are normalized with the controls made in the absence of BMP-2.

We also developed research to generate intrafilm fibronectin placement, for films with exceptionally high matrix protein content [2], FIG. 2.

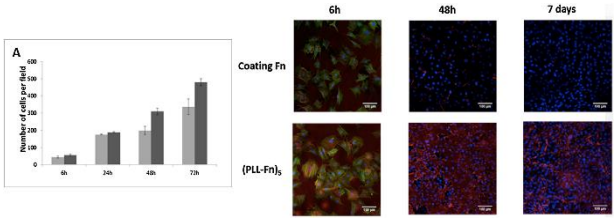

FIG. 2. (A) Cell adhesion and proliferation on the Fn monolayer (light grey) and the (PLL-Fn)5film (dark grey) after 6 h, 24 h, 48 h and 72 h. The experiment was performed three times independently, and the vertical lines represent the standard deviation. (B) Fn reorganization by MC3T3-E1 pre-osteoblastic cells. Cells were cultured for 6 h, 48 h and 7 days on either a Fn monolayer or a (PLL-Fn)5film, and stained for nuclei and actin. Fn was fluorescently labeled with Alexa Fluor 568.

These strategies represent appealing bioactive systems able to enhance cell adhesion, spreading, proliferation and differentiation, and offer great potential toward a variety of cell contacting applications, including antimicrobial.
On the other hand, we have proposed the engineering of a hydrogel scaffold glucose delivery system for enhancing mesenchymal stem cells survival. Such a system addresses a significant tissue engineering challenge: the massive death of transplanted cells that typically occurs following engraftment using currently available scaffolds. By supplying glucose in situ as a metabolic fuel for Mesenchymal Stem Cells (MSCs) in severe hypoxia, this new scaffold is shown to significantly enhance MSC survival.

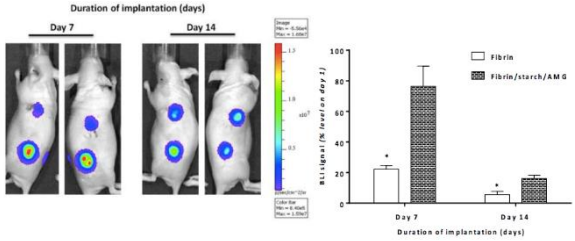

FIG. 3. Bioluminescence imaging and quantification of luciferase-labelled hMSCs loaded in fibrin and in fibrin/starch/AMG hydrogels, after subcutaneous implantation in nude mice.

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BIOACTIVE GLASSES: BENEFITS AND DRAWBACKS FOR USE IN BONE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 8]

Introduction

The first bioactive glass (45S5) was discovered in 1969 by L.L. Hench and since much work has focused on this unique material. Bioactive glass is the first synthetic material that demonstrates not only osteoconductivity but also osteoinductivity. The main advantage of bioactive glass lies in the ability to modify the composition in order to tailor the degradation kinetics, thus matching the rate of tissue regeneration. Unlike other synthetic materials (i.e. polymers, metal, ceramics), ions of therapeutic interest can be incorporated into the glass structure. For example, antimicrobial, angiogenesis or anti-inflammatory properties, just to cite a few, can be imparted to the bone graft by adding specific ions.

However, the use of bioactive glass is facing challenges. It is well known that an optimum bone graft, should be a 3D structure with high porosity (>60%), large pore size (>100µm) and mechanical properties at least similar to the cancellous bone. In order to process such architecture, ceramics and glasses are often sintered at high temperature. However, typical bioactive glasses are prone to crystallization during the sintering process, leading to a decrease, or even suppression of their bioactivity. Furthermore, glasses, per definition, are hard materials, leading to difficulties for surgeons to shape them on site.

Materials and Methods

In this presentation, an introduction to traditional bioactive glass will be provided, focusing on their advantage and disadvantage. Processing and characterization of new borosilicate glasses enabling sintering without adverse crystallization will be presented. The thermal properties of these new glasses were assessed by DTA and XRD. The dissolution mechanism of these glasses was quantified by pH change, ion release (using ICP-OES) and glass structural change (using FTIR). The ability of these glasses to produce a hydroxyapatite reactive layer was confirmed by SEM/EDX. The best glass candidate was 3D printed using a n-Scrypt 3D printer. The architecture (µCT) and dissolution in simulated body fluids were studied.

Human adipose Stem Cells were plated at the surface of glass disc and on 3D printed scaffolds. Cell proliferation was measured. Cell morphology was assessed by immunostaining. Osteogenic commitment was proven by culturing the cells in basic medium as well as osteogenic medium and tracking osteogenic markers.

Finally to facilitate shaping and processing, these new glasses were introduced in composites and hybrids biomaterials. Processing of PLA/bioactive glass and Gelatin/GPTMS/bioactive glass biomaterials as well as their cell/material interaction will be presented.

Results and Discussion

While typical silicate bioactive glasses have found space in clinics for the reconstruction of bone defect, sintering them into scaffolds leads to crystallization, in turns reducing their bioactivity. The new borosilicate glasses developed have shown to not only possess extended hot forming domain but also to promote early cells osteogenic commitment. Indeed, while the cell proliferation was reduced at the surface of the borosilicate glasses, compared to the silicate counterpart, up-regulation of osteogenic and endothelial markers was demonstrated. Borosilicate glasses were shaped into 3D scaffolds with controlled porosity and pore size using the porogen burn-off, 3D prototyping technique or supercritical CO_2 foaming when in composites (FIG. 1).

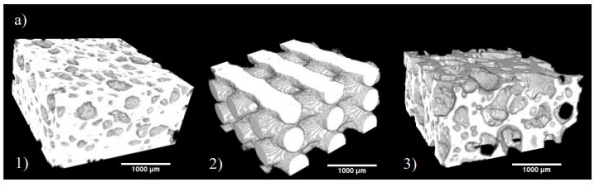

FIG. 1. Borosilicate glass scaffold processed using the porogen burn-off technique (1) 3D prototyping (2) and PLCL/bioactive glass scaffold processed via supercritical CO_2 foaming (3).

The developed scaffolds have mechanical properties superior to the cancellous bone, pore size >100µm and interconnection >50µm.

While these results are promising, the problem of shaping the scaffold on-site remain a drawback. Composites (PLCL/bioactive glasses or PLA/Bioactive glass) have, thus, been developed. PLA/borosilicate glasses composites were processed via twin-screw extrusion, in view of producing screw and/or plates. The presence of bioactive glasses was found to increase the rate of degradation of the PLA, while providing ions, leading to osteoinduction.

Finally, hybrids based on Gelatin / GPTMS / borosilicate glasses or Gelatin / Alginate / bioactive glasses were processed using a one-pot technique. The presence of the bioactive glass was found to stabilize the organic network. The first test of bioprinting (FIG. 2) was successful and cell culture will be continued.

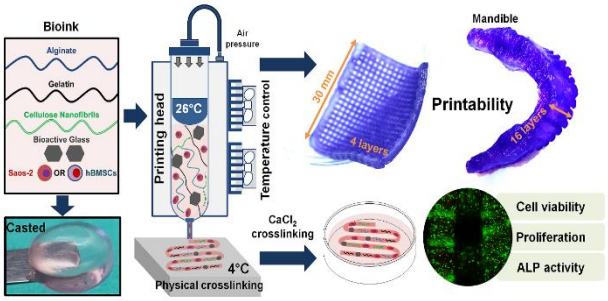

FIG. 2. Schematic of the bioprinting process [1].

Conclusions

In conclusion, bioactive glasses have the unique ability to induce osteoinduction as well as other beneficial therapeutic effects, without the need for costly, and sometimes toxic additional molecules. The composition of the glasses can be tailored in order to allow scaffold production with controlled pore size and structure.

Bioactive glasses can be introduced into polymeric matrix natural or synthetic to benefit from its osteoinductive properties.

Acknowledgments

I would like to thank all the members of my research group, as well as my collaborators from University of Cergy-Pontoise (France), Politecnico di Torino (Italy), University of Bergen (Norway). The Academy of Finland, the Jane and Aatos Erkko Foundation and EU-ITN-H2020 are acknowledge for financial support

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BACTERIAL CELLULOSE - HYDROXYAPATITE COMPOSITES DECORATED WITH SILVER NANOPARTICLES FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 9]

Introduction

Bacterial cellulose (BC) is a natural biopolymer with excellent properties, which recommend it as a component in a broad range of organic or hybrid composites [1], as well as a template in the synthesis of nanostructures with tunable properties [2].

Hydroxyapatite (HA) is a mineral phase widely used for medical applications that require both suitable mechanical properties and remarkable biological response when integrated in living bodies; it is approached either for repairing/replacing damaged hard tissues [3] or as bioactive coating on inert implants [4].

Metallic silver (MA) nanoparticles are considered a promising antibacterial agent, being frequently employed for the optimization of implants behaviour in terms of infection prevention [5,6].

In this context, complex composites based on BC membranes, HA phase and MA nanoparticles were produced by two different routes and subsequently characterized in order to demonstrate the potential of such materials to be integrated in the clinical use for hard tissue engineering.

Materials and Methods

The composite samples were synthesized by two different routes. The first one involves the mechanical mixing of blended BC membranes, laboratory synthesized HA powder and MA nanoparticles aqueous suspension. The second one implies the *in situ* deposition of HA phase on the blended BC membranes by immersion in precursor solution containing calcium and phosphate ions, as well as the addition of MA nanoparticles, followed by homogenisation. The weight ratio between BC and HA was set at 1:1, while MA loading was varied: 1, 2 and 5 wt% reported to the other two constituents. All masses were subjected to a freeze-drying procedure in order to preserve a porous *3D* structure.

The specimens were investigated from compositional, structural and morphological point of view through X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy EDX.

Results and Discussion

BC membranes with fibrillar and spongy appearance (FIG. 1) were obtained with the help of *Acetobacter* sp. strain isolated from traditionally fermented apple vinegar, purified in basic medium, thoroughly washed and then used for both synthesis routes in the blended form.

The preliminary preparation of HA powder and MA nanoparticles in the first case, as well as HA phase *in situ* deposition in the second one were proved through the presence of specific peaks in the XRD patterns.

FTIR spectroscopy was approached so as to assess the possible chemical interactions established at the interface between the three components.

FIG. 2 presents the microstructure of one of the final composites, demonstrating the strong adhesion of the mineral and metallic phases to the BC fibres. In addition, the EDX spectra allowed homogeneity to be ascertained throughout the entire sample volume.

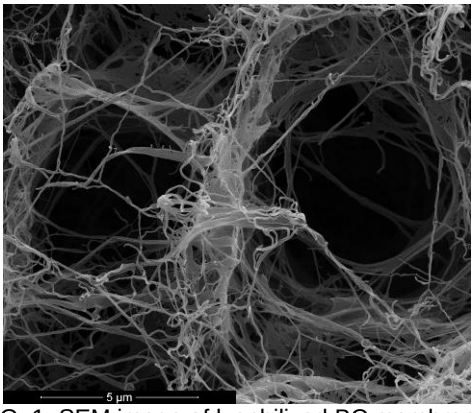

FIG. 1. SEM image of lyophilized BC membrane.

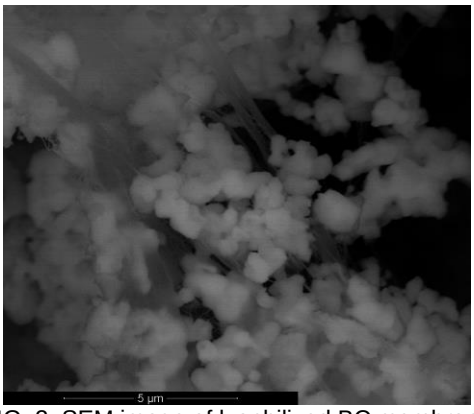

FIG. 2. SEM image of lyophilized BC membrane loaded with two inorganic phases.

Conclusions

Composite materials containing BC, HA and MA were successfully obtained by two straightforward methods. Such biomaterials, achieved by employing low cost precursors, represent important candidates for the development of bone scaffolds with antibacterial properties.

Acknowledgments

This work was financially supported by the project *Smart Scaffolds Built on Biocellulose 3D Architecture or Artificial Electrospun Templates for Hard Tissue Engineering* (ScaBiES), PN-III-P1-1.1-TE-2016-0871 (contract number 66/2018), financed by the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI).

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MECHANICAL AND ABSORPTION PROPERTIES OF COMMERCIAL HYDROGEL DRESSINGS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 10]

Introduction

Hydrogels have been described as essential biomaterials in the field of tissue engineering, regenerative medicine, and drug delivery applications due to their specific characteristics [1,2,4]. The contact time of hydrogel with patient environment has important influence on development of biofilm (bacterial adhesion) on biomaterial surface. The aim of this work was comparison of three different structures (foam, fibrous and solid) of hydrogels through the mechanical tensile test, wettability test and the fluid absorption ability in the context of durability.

Materials and Methods

The objects of the study were Aquacel Ag (ConvaTec), Granuflex (ConvaTec) and Aqua-Gel (Kikgel) dressings.
The tensile tests were conducted with the use of the MTS tensile machine with the rate of 10 mm/min in the room temperature (23±2ºC) until broken of specimens. Each group was represented by three samples. The measurement base of specimens was 100 mm, the width was 10 mm and thickness was respectively 1, 3 and 4 mm. The contact angle (Θ) values were measured with the use of sessile drop method by the See System computer-based instrument produced by Advex Instruments. The volume of liquids drops were 0.5 µl. Three measurements liquids: (W) distilled water (Poch), (D) diiodomethane (Merck) and (G) glycerin (Chempur) were used. The changes in surface free energy (γ_S SFE) and its components were estimated by using analytical van Oss-Chauhury-Good (vOCG) model. Fluid absorption ability of hydrogels was tested for two liquids: distilled water and 0.9% salt saline solution, in room temperature trough 0h, 2h and 24 h.

Results and Discussion

The load-displacement curves for all materials after unpacking were shown in FIG. 1. Different character of hydrogels structures impacts on behavior under load, that is important issue for wound dressing. The maximal load obtained in tensile test were respectively for Aquacel, Aqua-Gel and Granuflex: 2.37±0.22, 0.89±0.07, 5.06±0.21 [N].

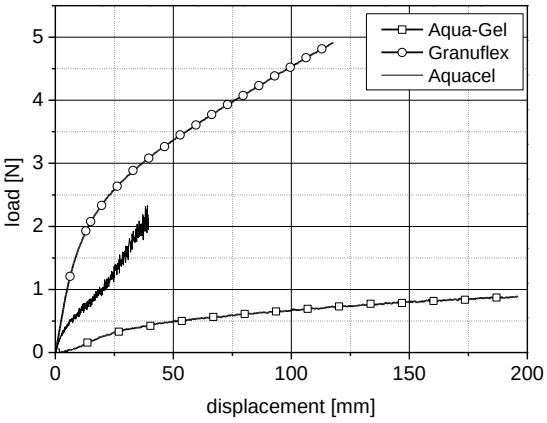

FIG. 1. Characteristics of tensile tests curves.

The instability of mass is shown in FIG. 2. The Aqua-Gel and Granuflex showed the stabilization of mass after ca. 7 days (in both liquids; the Aquacel has been defragmented in distilled water after 36 h, and in salt solution after 22 days. TABLES 1 and 2 present the values of contact angles and surface free energy. The decrease of contact angle of water was observed for Granuflex and Aqua-Gel.

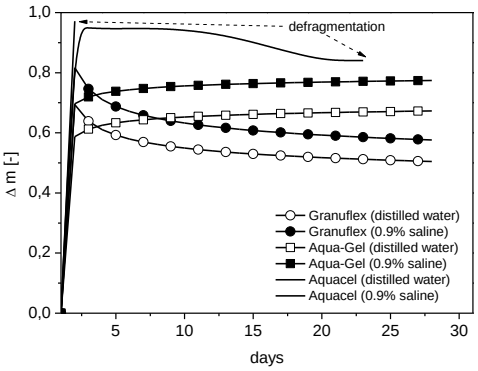

FIG. 2. Change in weight of hydrogels immersed in distilled water and 0.9% saline.

TABLE 1. The values of contact angles [°].

Time [h]	Material *	Θ_W	Θ_D	Θ_G
0	H2	108.1±7.9	60.1±7.6	101.8±6.7
2		79.8±3.1	62.7±2.8	77.18±3.1
24		67.2±6.2	47.6±7.1	58.8±10.3
0	H3 (-)	86.4±2.3	31.6±3.7	83.9±3.4
2		84.8±3.0	37.5±8.4	84.1±3.0
24		74.0±2.9	43.2±2.0	78.3±5.0
0	H3 (+)	107.2±6.9	61.5±4.2	108.4±8.9
2		100.4±6.9	51.47±8.9	89.0±6.9
24		88.4±11.7	63.7±4.1	92.7±5.3

*H1 – Aquacel (for H1 was no possibility to realize the wettability test), H2 - Aqua-Gel, H3- Granuflex (+/-) side with/without glue (inside/outside)

TABLE 2. The values of SFE and its components [mJ/m^2] for the surface of hydrogel dressings.

Time [h]	Material *	γ_S	γ_s^d	γ_s^p	γ_s^+	γ_s^-
0	H2	30.68	28.52	2.16	1.05	1.11
2		28.85	27.01	1.84	0.07	11.62
24		41.93	35.57	6.35	0.78	12.96
0	H3 (-)	49.47	43.55	5.91	1.23	7.14
2		47.10	40.85	6.25	1.09	8.90
24		44.59	37.95	6.64	0.60	18.25
0	H3 (+)	33.80	27.72	6.08	2.70	3.42
2		34.26	33.45	0.81	0.17	0.95
24		32.39	26.43	5.96	0.72	12.29

Conclusions

The results showed dispersive character of hydrogel surface in initial state, what is similar to the pig's skin [3], as well as good level of stability of properties necessary to the security wound in healing phases.

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ELECTROPHORETIC DEPOSITION OF A METAL-CERAMIC COATING CONTAINING MCrAlY (M:Ni,Fe) BOND COAT FOR CARDIOVASCULAR IMPLANTS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 11]

Introduction
The main aim of the presented project is the development of a reproducible, tailor-made metal-ceramic coating with hemocompatible properties for permanent medical use on cardiovascular implants with direct blood contact such as stents. In addition to hemocompatibility, the coating should increase the lifetime of the applied materials. The metal matrix provides the necessary flexibility and adaptation of the expansion to the substrate. The dispersed ceramic particles in the metallic matrix improve the hemocompatible properties of the coating [1-3].

Experimental Methods
In this work, MCrAlY (M: Ni, Fe) bond coat and Al-Al_2O_3 top coat were applied using electrophoretic deposition and sintering. Using the electrophoretic deposition and the charging system of iodine-acetone, NiCrAlY and FeCrAlY bond coat was successfully deposited on a steel substrate. The samples were sintered in argon and vacuum ($1x10^{-5}$ mbar) at the range of temperatures from 1000°C up to 1200°C for 30 and 60 minutes. In order to improve the adhesion, the steel plates were previously sandblasted. The Al-Al_2O_3 composite coating was applied as a second layer by the electrophoretic deposition and the charging system of iodine-acetone and sintering, as well. To evaluate the phases formed at each temperature the XRD-analysis was performed. The morphology of the samples was analyzed by SEM and an adhesion test was executed.

Results and Discussion
In this study, we could show that the MCrAlY (M:Ni,Fe)-coatings could be successfully obtained by the electrophoretic deposition by using the charging system of iodine-acetone without any other additives. It is also possible to perform the sintering in argon or vacuum atmosphere with good adhesion to substrate. The top coat (Al-Al_2O_3) adheres the bond coat in a proper way. Hemocompatibility tests according to ISO 10993-4 are ongoing.

Conclusion
MCrAlY (M:Ni,Fe) is a trendy and promising material that can provide good adhesion of ceramic and metal and can be applied by a simpler method like the electrophoretic deposition. It can be used not only as thermal barrier coating but also as a bond coat for ceramic coatings for medical use.

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THE BIOLOGICAL DEGRADATION OF PURE BONE CEMENT AND BONE CEMENT WITH NANOMETALS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 12]

Introduction

Bone cement is a biomaterial widely used in various fields of medicine. Its main tasks are bonding and stabilizing implants with bone and as a filler of bone defects and spaces between the implant and the bone. It is characterized by biocompatibility and good mechanical properties, as well as it is easy to form. Thanks to the use of bone cement, better cell adhesion to the implant and osseointegration can be obtained.

The paper concerns pure bone cement and bone cement with the addition of nanometals. The samples were placed into a bacterial liquid (generated by one of the researchers) for 1,3 and 6 months. This liquid contains the five most common bacteria in the operating theatre. After that, it was examined by the scanning electron microscope and biological microscope.

The work aims to select the best material, so that in order to prevent bacterial adhesion and thus to counteract infection. The effect of bactericidal additives on bacterial adhesion and biofilm formation was also determined.

Materials and Methods

The samples were made of pure bone cement and with the addition of nanometals (silver, copper).

The samples were placed into a bacterial solution consisting of the 5 most common bacteria in the operating room, for a period of 1,3 and 6 months. After that the macroscopic observation was performed with the use of ZEISS biological microscope AXIO Observer.D1 (FIG. 1).

FIG. 1. The biological microscope Zeiss.

Results and Discussion

Microbial adhesion is the initial step in colonization and the formation of a biofilm – accumulated biomass of microorganisms and extracellular materials on a solid surface. Biofilms can be detrimental to both human life and industrial processes, causing infection associated with medical implants, pathogen interaction with host cells, periodontitis or dental caries, contamination of food from processing equipment, enhancement of metal corrosion, formation of marine biofilms on ships` hulls, and so on [1].

Biological tests on pure bone cement after 1 month in the bacteria liquid revealed single bacteria. However, after 6 months the surface was covered with biofilms (FIG. 2).

Bone cement with nanometals after 1 and 3 months did not show any bacteria on the surface. After 6 months there were single bacteria on nanosilver samples (FIG. 3).

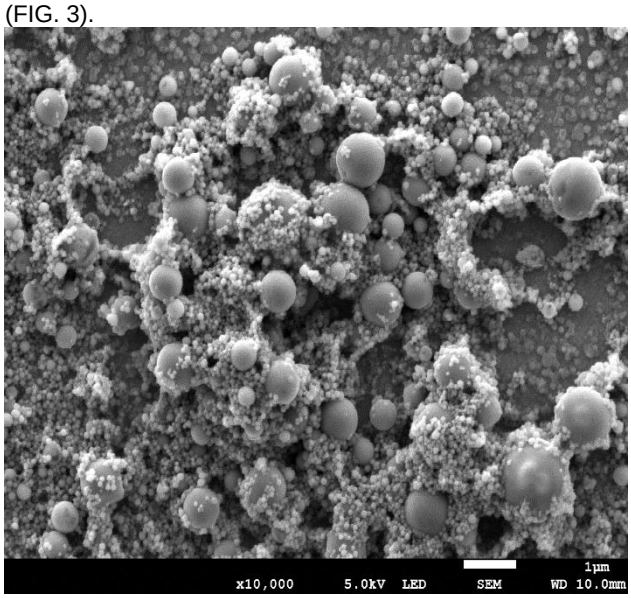

FIG. 2. Pure bone cement after 6 months staying in bacteria liquid.

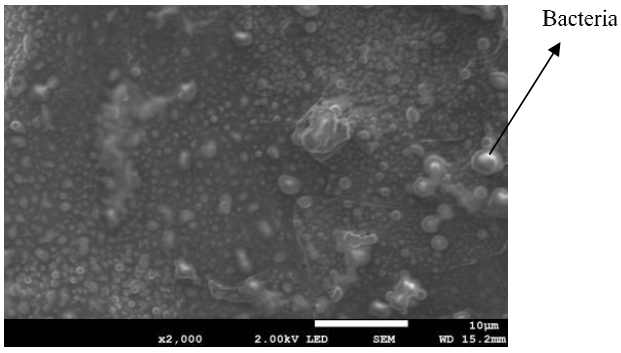

FIG. 3. Bone cement with silver nanoparticles after 6 months staying in bacteria liquid.

Conclusions

The paper shows, that nanoparticles can be used in bone cement to prevent infections. The results of the research prove, that nanoparticles are the alternatives to antibiotics, whose activity is gradually decreasing as a consequence of the rise in antibiotic – resistant microorganisms. Furthermore, the nanoparticles are effective also against bacterial strains already resistant to some of the common antibiotics used in bone cements.

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FORMATION OF BACTERIOSTATIC COATINGS OF Ti ALLOY IMPLANTS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 13]

Introduction

Titanium is widely used as long-term material for implantation [1,2]. Titanium alloys such as Ti-6Al-7Nb, Ti-13Nb-13Zr or gum-metal type Ti-2Ta-3Zr-36Nb exhibit mechanical properties close to a natural part of bone. Multi phases titanium alloys also are able for surface treatment [3]. Plasma electrolytic oxidation process allows formation of a porous oxide layer. However, the sensitive compounds like antibiotics cannot be incorporated into layer in their active form. Formation of hybrid oxide-polymer layer is a proposition for functionalization of titanium alloys to obtain bacteriostatic and/or surface with bactericidal properties.

Materials and Methods

Titanium alloy (Ti-2Ta-3Zr-36Nb) was anodized in a 0.1 M $Ca(H_2PO_2)_2$ solution at 300 V. All the conditions applied for surface treatment was presented in paper [4]. Poly(sebacic anhydride) (PSBA) layer was formed on previously anodized Ti alloy. The polymeric coating was loaded with cefazolin. Morphology, degradation of the layer were evaluated using a scanning electron microscope (Phenom, ProX, accelerating voltage 5kV) equipped with 3D surface roughness reconstruction software. HPLC technique (Merch-Hitachi chromatograph) was applied to determine concentration of the loaded drug and its stability in solution. The samples were immersed in artificial saliva at 37°C up to 10 h. The activity of the drug released from the coatings, as well as adhesion of the bacteria on the surfaces was determined using references *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 700296) and clinical *S. aureus* (MRSA 1030), and *S. epidermidis* (15560) strains.

Results and Discussion

FIG. 1A presents the representative surface of Ti alloy after plasma electrolytic oxidation. A porous microstructure of the layer was a result of the anodization process. The average surface roughness of the hybrid coatings was between 0.78 µm ±0.17 - 0.98 µm ±0.12. The determined concentration of cefazolin loaded in hybrid coating was 17.62 µg/cm^2 ±0.05. The average concentration of the drug in artificial saliva collected up to 10 h of material degradation is presented in FIG. 1B. During first hours of material immersion more than 20% of loaded drug was released to artificial saliva. ^{1}H NMR analysis showed that the PSBA was degraded in 77.8 mol.% after 7 days of material immersion.

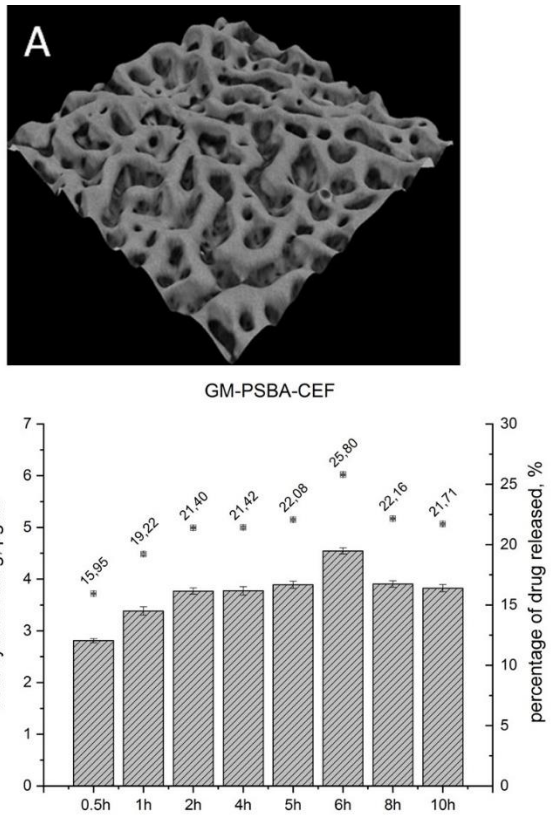

FIG. 1. The representative surface of Ti alloy after plasma electrolytic oxidation (1A); Average concentration of the drug in artificial saliva collected up to 10 h of material degradation (1B).

The minimal inhibitory concentration (MIC) was determined for cefazolin released after 30 min (2.81 µg/ml) of material immersion for reference bacteria strains. Growth of clinical bacteria was inhibited when the concentration of cefazolin increased up to 4.5 µg/ml. Amount of drug released from the coatings was enough to inhibit adhesion of reference and clinical bacteria strains as well.

Conclusions

Anodized Ti alloy surface is favourable for formation of a hybrid, oxide-polymer layer. Poly(sebacic anhydride) was deposited onto the anodized Ti without losing its characteristic microstructure. The cefazolin loaded in polymeric layer was stable and its concentration was enough to inhibit reference and clinical bacteria strains on surfaces.

Acknowledgments

This work was supported by the National Science Centre, Poland (UMO-2016/21/D/ST5/01652).

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE ANALYSIS OF DRUGS RELEASED FROM POLYMERIC COATINGS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 14]

Introduction
Liquid chromatography is a separation method that allows simultaneous separation and determination of a large number of compounds in many different matrixes. The possibility of using different detectors increases the range of applicability and usefulness of the method. Analytical procedures using HPLC have been applied in many fields: criminalistics, pharmacy, food industry, environmental protection, etc.

Materials and Methods
The study included drugs from the group of antibiotics: amoxicillin (AMX), cefazolin (CEF) and vancomycin (VANKO). The chromatographic system was applied in which the C18 (150x4.6, 5um) (TOSOH, Bioscience) stationary phase was used. The mobile phase was a mixture of 0.05% trifluoroacetic acid solution in water with acetonitrile. The elution was carried out in both isocratic and gradient systems. Detection was carried out using spectrophotometric diode array (DAD) and fluorescence (FL) detectors. The samples for the study were solutions of drugs released from polymeric coatings (PLGA) into the model matrix (artificial saliva and artificial inflammatory saliva). The studies were carried out at different times of drug release as well as in order to determine the total amount of drug applied.

Results and Discussion
Chromatographic systems for AMX, CEF and VANKO determinations were developed. These systems allow to conduct research with both single compound coatings and mixtures of these drugs. The developed procedures were validated and simultaneously studies on the stability of analytes in various conditions were conducted. The developed analytical procedures made it possible to monitor not only the drug release from the polymer film by determining its concentration in the solution, but also to track the stability of the active compound during its release to the model solution. Stability studies were carried out in model systems in various concentrations of analytes for up to 30 hours. It was found that the analyses were stable during the release of saliva to the model solution even up to 30 hours. In the case of studies with the model solution of saliva in inflammation, it was found that AMX decomposes after only 4 hours in the solution, CEF decomposes after 24 hours in the solution, while vancomycin showed stability throughout the experiment.

Conclusions
High-performance liquid chromatography systems for identification and determination of drugs and their derivatives released from polymeric coatings were developed. The use of high-performance liquid chromatography in studies on the release of drugs from polymeric coatings allows full monitoring of the drug during the experiment. Compared to other analytical techniques (such as UV / VIS spectrophotometry), it not only determines the content of the analyte in the solution, but also whether there are any side reactions during the release affecting the chemical form of the drug.

Acknowledgments
This work was supported by the National Science Centre, Poland (UMO-2016/21/D/ST5/01652).

EFFECT OF PLCL/PCL NANOFIBERS-BASED SCAFFOLDS WITH FIBRIN ASSEMBLY CONTAINING PLATELET LYSATE ON SKIN CELLS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 15]

Introduction

Skin wound healing is a process that involves several cell types, such as keratinocytes, fibroblasts, endothelial cells and other immune cells. The ability of these cells to respond upon injury is critical to start the process of healing. However, skin regeneration could be reduced due to several factors including people aging and diabetes [1]. Chronic wounds are affecting millions of patients around the world and became a serious problem for health services in developed countries [2]. In this regard, the development of scaffolds and wound dressings that improve skin regeneration is necessary. Here, we show the effect of PLCL/PCL nanofibers scaffolds that have been assembled with fibrin containing platelet lysate on keratinocytes.

Materials and Methods

PLCL/PCL nanofibers were coated with fibrin assemblies with different content of platelet lysate (1%, 5%, 10%, 20%, 50%, 100% and control 0%). Human keratinocytes cell line (HaCaT cells) were cultured directly on samples to evaluate the cytocompatibility and the effect of platelet lysate on cell growth and differentiation. Cell proliferation was quantified after 1, 4, 7 and 14 days in culture using MTS assay kit (Abcam) and the results were statistically evaluated using one-way analysis of variance (ANOVA) and Bonferroni multiple comparison test (SigmaStat). Cell adhesion and F-actin cytoskeleton organization after 24h in culture were analysed by actin fibres staining with phalloidin-TRIC and visualized with confocal laser scanning microscope (CLSM) on different z-stacks. Intermediate filaments presence and assembly were evaluated by staining two different types of cytokeratin markers. Cytokeratin 14 is a marker of basal keratinocytes, whereas cytokeratin 10 is a marker of stratified differentiation. Both cytokeratins were stained using immunodetection and visualized with CLSM.

Results and Discussion

Results indicated that keratinocytes were able to adhere and grow directly on PLCL/PCL nanofibers scaffolds. After 24h in culture, randomly distributed cells were detected on samples and the cells showed a rounded shape morphology similar in all samples analysed. After 14 days in culture, differences in cell metabolic activity were observed on samples containing 50% and 100% of platelet lysate. The increase in platelet lysate content improved cell proliferation which demonstrated the safety of the polymer and the positive effect of the platelet lysate. In addition, the second piece of PLCL/PCL nanofibers-based scaffolds were added on day 7 days to compare the effect of using 1 or 2 scaffolds on cell proliferation. The results clearly indicated that the presence of the second scaffold in medium increased cell metabolic activity and proliferation in the most samples.
Images of cytokeratin 10 and 14 immunostaining showed an increased number of differentiated cells positive for cytokeratin 10 on PLCL/PCL nanofibers scaffolds with a high percentage of platelet lysate. Keratinocytes showing cytokeratin 10 were detected on the upper layers of cells (FIG. 1) in agreement with other authors that evaluated the presence of cytokeratin types according to the differentiation of stratified layers of the skin [3]. Results obtained from adhesion and proliferation are in agreement with the differentiation results, which indicate the positive effect of platelet lysate in keratinocytes regeneration. Previously, the effect of platelet lysate on HaCaT cells has been studied [4]. In the present work, we also analysed the effect of platelet lysate incorporated in fibrin assemblies on nanofiber scaffolds with interesting results on keratinocytes.

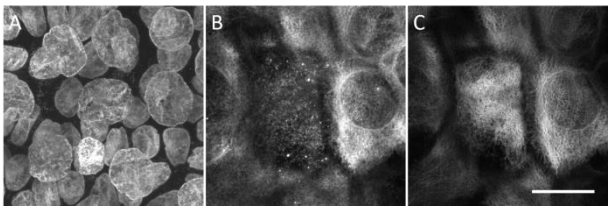

FIG. 1. Immunofluorescence staining of HaCaT cells after 7 days in culture grown on PLCL/PCL nanofibers scaffolds with fibrin assembly containing 100% of platelet lysate. Images of nuclei (A), cytokeratin 14 (B) and cytokeratin 10 (C) are shown.

Conclusions

Keratinocytes were able to spread and proliferate on PLCL/PCL nanofibers scaffolds, and the increase of platelet lysate content in fibrin assemblies on the samples improved the proliferation rate and the differentiation of keratinocytes. Both scaffold chemical properties and its biocompatibility and bioactivity in experiments on keratinocytes allow considering it to be a promising scaffold for skin wound healing. In addition, future experiments will be performed to evaluate the effect on other cell types present on skin wound.

Acknowledgments

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NEAR-FIELD ELECTROSPINNING OF POLYDIOXANONE TISSUE REGENERATION TEMPLATES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 16]

Introduction

The ideal biodegradable, biomaterial-based regeneration template should be engineered to mimic the extracellular matrix (ECM) of native tissues to coordinate the cellular response and ultimately guide *in situ* regeneration. Electrospinning is a popular method to artificially recreate the ECM, and by reducing the process's working distance to a few millimeters, the method of near-field electrospinning (NFES) was devised[1]. NFES allows for the "direct writing" of fibers; thus, adding another dimension of tissue template specificity and tailorability. Specifically, mechanical properties, pore size, and fiber orientation can be tightly controlled to bring about desired cellular responses. With this project, we demonstrated NFES devices designed around two commercial 3D printers for the creation of highly precise tissue regeneration templates.

Materials and Methods

A preliminary NFES apparatus was designed around a MakerFarm Prusa i3v 3D printer based on the work of Fattahi et al[2]. The stock filament extruder was replaced with a custom 3D printed adapter to accommodate a NE-300 Just Infusion™ syringe pump. Fibers could be directly written onto a flat collector and sequential stacked to create 3D, fibrous constructs. The polymer polydioxanone (PDO) was chosen as a candidate material due to its superior inflammatory response, mechanical properties, and *in vivo* degradation rate of 6-8 weeks. PDO solutions were made at varying concentrations in 1,1,1,3,3,3-hexafluoro-2-propanol. Template sheets were created by sequentially writing parallel fibers with a 90° rotational offset after each layer. The effect of polymer concentration on fiber size was evaluated by varying PDO concentration from 140 to 220 mg/mL in increments of 20 mg/mL. The remaining processing parameters of air gap, applied voltage, translational velocity, polymer flow rate, and needle gauge were held constant at 1.8 mm, -1.3 kV, 30 mm/s, 15 µL/hr, and 23, respectively. Scaffolds were imaged with a Nova Nano 650 FEG scanning electron microscope, and fiber diameters were measured via Fibraquant v1.3.149. Data were analysed non-parametrically by Kruskal-Wallis test with Dunn's multiple comparison in Prism 7.

Subsequently, a successor apparatus was constructed around a MakerFarm Pegasus 12" 3D printer with a Legato 130 syringe pump (KD Scientific) to create regeneration templates with more complex 3D geometries. The Pegasus 3D printer was integrated with a rotating cylindrical mandrel driven by a stepper motor (Applied Motion Products). This platform allowed for a fiber to be written onto a cylindrical collector with a wind angle as the resultant vector between the translational 3D printer and rotational mandrel. Cylindrical templates were created by translating the 3D printer at a velocity of 70.7 mm/s and rotating the mandrel at an outer surface velocity of 70.7 mm/s, producing a 45° wind angle from the center axis with a resultant velocity of 100 mm/s. Air gap was held constant at 1.8 mm, applied voltage at +1.4 kV, polymer concentration at 120 mg/mL, flow rate at 25 µL/hr, and a needle gauge of 26.

Results and Discussion

As early stage preliminary data, the direct writing of PDO fibers resulted in orderly sheet templates with tailored fiber sizes (FIG. 1 A, B). The template's average fiber diameter significantly increased from 4.1 ± 1.1 to 8.0 ± 2.1 µm over the PDO concentration range of 140 - 220 mg/mL ($p < 0.05$) (FIG. 2). The addition of a rotational collecting surface resulted in the creation of complex cylindrical templates with an average wind angle of 46.9° ± 6.9°, fiber sizes of 2.0 ± 1.1 µm and pores sizes of 17.7 ± 7.7µm (FIG. 3 A, B).

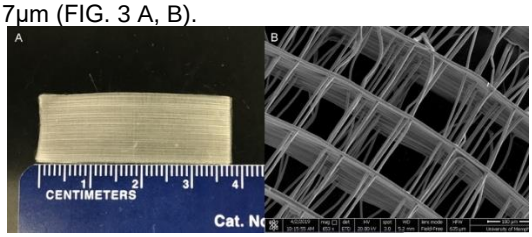

FIG. 1. A. Sheet template.; B. SEM of sheet template with fiber diameter of 4.9 ± 1.5 µm and pore size of 116 ± 34 µm. SEM scale bar 100 µm.

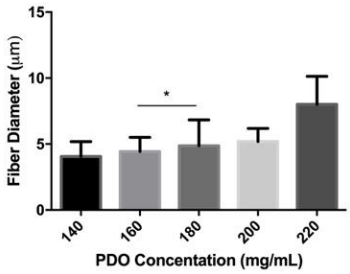

FIG. 2. Fiber diameter as a function of PDO concentration (n=5). All comparisons significant ($p < 0.05$) except *.

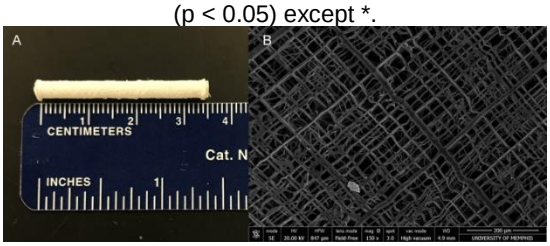

FIG. 3. A. Cylindrical tissue regeneration template; B SEM of cylindrical template with fiber diameter of 2.3 ± 1.3 µm and pore size of 19.8 ± 10.8 µm. SEM scale bar 200 µm.

Conclusions

We have demonstrated that NFES of PDO is a viable technique to precisely create multiple types of 3D, fibrous tissue regeneration templates. The creation of seamless templates has numerous biomedical applications in fields such as vascular, neural, gastrointestinal, and urinary tissue engineering.

Future Work

The mechanical properties of these templates will be evaluated as a function of processing properties and design geometry. Furthermore, these materials interactions with the innate immune system, most notably the neutrophil, will be characterized.

Acknowledgments

The authors thank the Memphis Institute for Regenerative Medicine (MIRM) for funding this project.

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HIGHLY MACROPOROUS CHITOSAN/AGAROSE/HA BONE SCAFFOLD PRODUCED BY COMBINATION OF FREEZE-DRYING WITH GAS-FOAMING AGENT

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[ENGINEERING OF BIOMATERIALS 153 (2019) 17]

Introduction

Typical bone scaffolds have a three-dimensional (3D) porous structure, which provides mechanical support and space for migration and proliferation of osteoblasts as well as mesenchymal stem cells. Importantly, open and interconnected porosity have the most significant biomedical importance since they facilitate bone tissue ingrowth and new blood vessel formation [1]. The aim of this study was to simultaneously apply freeze-drying method with a foaming agent to produce biocompatible and highly macroporous chitosan/agarose/nanoHA biomaterial for bone tissue engineering applications.

Materials and Methods

Biomaterial fabrication. Bone scaffold made of chitosan/agarose matrix reinforced with hydroxyapatite nanopowder (nanoHA) was produced using sodium bicarbonate as a source of CO_2 and freeze-drying method (Polish patent application no. P.426788). The resultant sample, marked as chit/aga/nanoHA, was made of 2% chitosan, 5% agarose, and 40% nanoHA.

Porosity determination. The porosity was evaluated by microcomputed tomography (µCT). CTAnalyser software (Bruker microCT) was used to determine pores diameter as well as total, open and closed porosity.

Biodegradation test. *In vitro* biodegradation test was performed in an enzymatic solution [2] and in citric acid (pH=3) and Tris-HCl (pH=7.4) buffer according to the international procedure for ceramic materials described in ISO 10993-14. Degradation of polysaccharides was determined by detection of reducing sugars, whereas degradation of ceramic component by assessment of the concentrations of Ca^{2+} and HPO_4^{2-} ions.

***Biocompatibility tests*.** The study was conducted using mouse calvarial preosteoblasts (MC3T3-E1 Subclone 4) and mesenchymal stem cells. The cytotoxicity of the biomaterial was assessed according to ISO 10993-5:2009 by agar diffusion indirect test. Osteoblast growth on the biomaterial was visualized by fluorescent staining of cell nuclei (DAPI) and cytoskeleton (AlexaFluor635phalloidin). Osteogenic differentiation on the scaffold surface was evaluated using mesenchymal stem cells by ELISAs and immunofluorescent staining.

Bioactivity test. *In vitro* apatite-forming ability assay was conducted in accordance with ISO 23317 procedure (2014). Briefly, bone scaffolds were immersed in the simulated body fluid (SBF) for 28 days and then the samples were analysed using SEM equipped with EDS detector to calculate the Ca/P atomic ratio (confirmation the presence of apatite crystals).

Results and Discussion

Produced scaffold exhibited highly macroporous structure (total porosity 63%) with a high share of macropores with diameter in the range 100-410 µm (FIG. 1, TABLE 1). According to available literature, macroporous structure of biomaterial provides good osseointegration, vascularization, and oxygenation of the implant *in vivo* [3,4]. Biomaterial was prone to enzymatic degradation, degradation in acidified microenvironment (e.g. osteoclast-mediated), and slow degradation under physiological pH of 7.4. Biocompatibility tests showed that novel scaffold was non-toxic, favoured osteoblasts growth (FIG. 2), promoted osteogenic differentiation, and induced apatite formation, indicating its high bioactivity which is essential for good implant osseointegration.

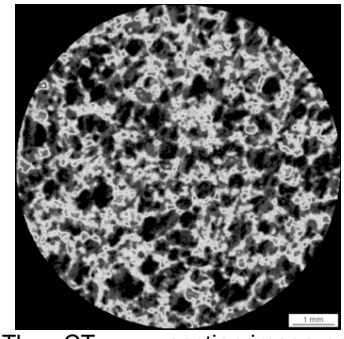

FIG. 1. The µCT cross-section image presenting microstructure of the chit/aga/nanoHA scaffold.

TABLE 1. Porosity of the scaffold assessed using µCT.

Type of porosity		
closed [%]	**open [%]**	**total [%]**
25.80	37.24	60.05

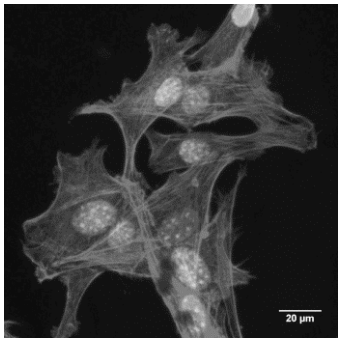

FIG. 2. Confocal microscope image of MC3T3-E1 cells cultured on the biomaterial.

Conclusions

Produced by novel method macroporous biomaterial has great potential to be used in regenerative medicine for acceleration of bone healing process.

Acknowledgments

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IN VITRO EVALUATION OF CELLULAR RESPONSE TO NOVEL AGAROSE/CHITOSAN/HA SCAFFOLD

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[ENGINEERING OF BIOMATERIALS 153 (2019) 18]

Introduction

Mechanical properties, roughness, wettability, functional groups, ion release and charge of surface scaffold are considered as relevant factors, having impact on cell behaviour (including attachment, proliferation, differentiation, and formation of an extracellular matrix – ECM). It is well known, that evaluation of cells' response to biomaterials under *in vitro* environment allows for better understanding of potential host-implant response under *in vivo* conditions [1,2]. The aim of this study was to evaluate cytotoxicity, adhesion, and proliferation of osteoblast cells and osteogenic differentiation of mesenchymal stem cells on the surface of developed novel scaffold for bone tissue engineering applications.

Materials and Methods

Preparation of scaffolds

The composition and production method of chitosan/agarose/nanohydroxyapatite (marked as chit/aga/HA) is protected by Polish Patent application no. P.426788. Briefly, chit/aga/HA was fabricated by mixing of chitosan (2 wt.%) and agarose (5 wt.%) suspension in acetic acid with hydroxyapatite nanopowder (70 wt.%) and sodium bicarbonate. Obtained paste was subjected to heating, then the sample was cooled and frozen. Frozen sample was lyophilised, followed by its neutralization in sodium hydroxide and finally left to dry in air. Microstructure of chit/aga/HA was visualized by stereoscopic microscope (Olympus SZ61TR) (FIG. 1).

Cell culture experiments

The cytotoxicity, adhesion and proliferation tests were carried out using normal human foetal osteoblast cell line (hFOB 1.19; ATCC). The cytotoxicity of the scaffold was evaluated by indirect test (MTT assay) using fluid extracts prepared in accordance with ISO 10993-5 (2009). Cell viability on the surface of the scaffold was assessed by fluorescent staining using Live/Dead Staining Kit. Cell adhesion and proliferation was determined by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin. The number of cells on the surface of the scaffold was estimated via counting of nuclei using ImageJ software. Stained cells were analysed under confocal laser scanning microscope (CLSM, Olympus Fluoview equipped with FV1000). The osteogenic differentiation was conducted using human bone marrow-derived mesenchymal stem cells (BMDSCs; ATCC). BMDSCs were seeded directly on the scaffold and cultured in osteogenic medium for 21 days. On the 3rd, 7th, and 21st day, collagen type I (Col I), bone alkaline phosphatase (bALP) and osteocalcin (OC) level in the cell lysates were determined using ELISAs.

Results and Discussion

MTT assay showed that osteoblast viability was near 100% compared to the control cells. Also, confocal microscope visualization confirmed that chit/aga/HA biomaterial is non-toxic. Additionally, fluorescent staining of cells on the surface of the scaffold showed that new biomaterial is favourable to cell adhesion since cells were well spread and had lengthened shape (FIG. 2). Cell proliferation experiment revealed good growth and proliferation of hFOB 1.19 cells on the scaffold surface as during 9-day cultivation, amount of cell number increased from 2 x 10^4 to 10.77 x 10^4 cells per cm^2 of the sample. Osteogenic differentiation assessment revealed that BMDSCs cultured on the scaffold synthesised osteogenic markers what confirmed osteoinductive ability of developed biomaterial (TABLE 1).

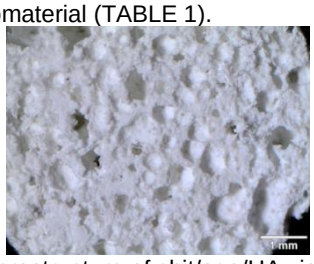

FIG. 1. Microstructure of chit/aga/HA visualized by stereoscopic microscope (scale bar = 1 mm).

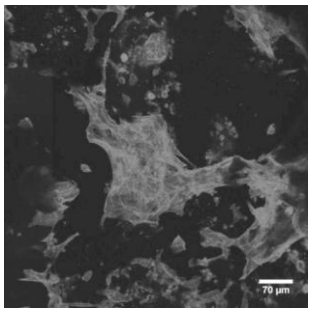

FIG. 2. Fluorescent staining of hFOB 1.19 cells growing on the surface of the chit/aga/HA after 9-day culture (magn. 200x, scale bar = 70 µm).

TABLE 1. Production of osteogenic differentiation markers by BMDSCs cultured on polystyrene (PS) and on the chit/aga/HA scaffold.

Time [day]	*Sample*	*Col I [ng/ml]*	*bALP [ng/ml]*	*OC [ng/ml]*
3	PS	10.62	0.09	0.98
	chit/aga/HA	4.88*	0.17*	2.36*
7	PS	6.11	1.15	4.34
	chit/aga/HA	8.26*	0.43*	7.27*
21	PS	7.40	4.46	15.36
	chit/aga/HA	8.84	0.19*	11.99*

*statistically significant results compared to PS, $P < 0.05$, unpaired t-test

Conclusions

Obtained results showed that fabricated scaffold is characterized by high biocompatibility. The surface of chit/aga/HA supports osteoblast adhesion, spreading and proliferation. Furthermore, novel scaffold has osteopromotive properties as it slightly enhances BMDSCs differentiation into osteoblastic lineage. This indicates that fabricated scaffold is a promising candidate for bone tissue engineering application.

Acknowledgments

The study was supported by the 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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STUDY OF LIPID-COPOLYMER SYSTEMS BY CRYO-TEM AND DLS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 19]

Introduction

Self-assembled structures of lipids and polymers, i.e. cubosomes, hexosomes or chimeric liposomes have increased research interest because of their potential to serve as biocompatible carriers in drug delivery systems, to increase drug solubilisation and allow to control the release of the payload [1]. The most popular structures reported in recent years are glyceryl monooleate (GMO) and phytantriol (PHYT) stabilized by PEO_{99}-PPO_{67}-PEO_{99} triblock copolymer (P407) [1,2].
In this work the formulation of aggregated structures of lipids (e.g. GMO, DPPC, PHYT) and polymers (e.g. Poloxamer P407 (PEO98-PPO67-PEO98), poly (ethylene oxide)–b–poly(ε-caprolactone) were studied (FIG. 1).

Materials and Methods

Hexosomes and cubosomes were prepared by Bottom Up Method and Top Down Method, For chimeric liposomes the thin film hydration method was used.
The morphology of prepared systems was studied by cryogenic transmission electron microscopy (cryo-TEM) using a Tecnai F20 X TWIN microscope (FEI Company) equipped with field emission gun, operating at an acceleration voltage of 200 kV. The hydrodynamic radius (Rh) and the size dispersity (PDI) of the prepared nanosystems were measured by dynamic light scattering (DLS).

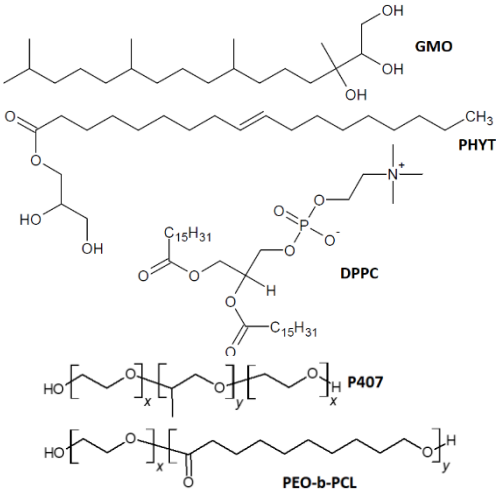

FIG. 1. Chemical structures of some used lipids and polymers.

Results and Discussion

The method of preparation, nature and amount of lipid and block copolymer used to form lipid-polymer structures, dictates the morphology of the resulting objects. There is a gamut of complementary techniques for characterization of the particles [3] (DLS, XRD, SAXS, m-DSC). Cryo-TEM can be distinguished because it allows for morphological visualization at near native state. Cubosomes of GMO:P407 9:1 had a hierarchically ordered internal structure, as shown in FIG. 2. Bicontinuous cubic (Q_{II}) phase was confirmed by FFT patterns.
For all studied lipid-copolymes systems cryo-TEM revealed the coexistence of different categories of structures with different grades of organization, including vesicles with no internal structure and more intricate, liquid crystalline confined nanoparticles. The comprehensive characterization is possible by a combination of cryo-TEM with Dynamic Light Scaterring as is demonstrated for chimeric liposomes in FIG. 3.

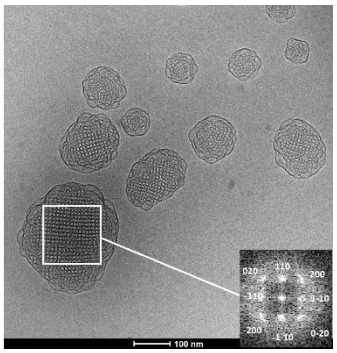

FIG. 2. Cryo-TEM image of GMO:P407 9:1.

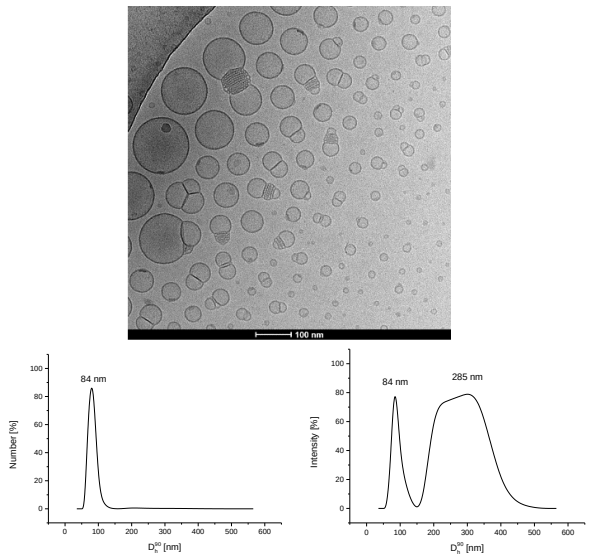

FIG. 3. Cryo-TEM image and size distributions from DLS of GMO-PDMAEMA-b-PLMA 9:1.

Conclusions

The aim of this study was to combine different techniques in order to characterize more comprehensively lipid-copolymer structures with a different architectures and compositions. We used cryo-TEM and DLS to examine the impact of block copolymers for lipid based particles. These techniques resulted to be effective in studying the case of lipid-copolymer systems.

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NOVEL HIGHLY BIOCOMPATIBLE CHITOSAN/AGAROSE FILM FOR POTENTIAL APPLICATION AS SKIN SUBSTITUTE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 20]

Introduction

Modern dermal substitutes applied in skin defects treatment should allow gaseous exchanges, remove excess exudates, preserve clammy environment at the wound interface and act as an external barrier to microorganisms. It should also be non-allergenic, non-toxic and promote wound healing [1]. It was shown that chitosan-based biomaterials have high biomedical potential for tissue regeneration [2]. The aim of the present work was to develop a brand-new biodegradable chitosan/agarose matrix-based film for potential skin tissue engineering applications.

Materials and Methods

Chitosan/agarose-based film was prepared by mixing agarose solution made by heating agarose powder in sodium hydroxide and chitosan solution prepared by dissolving chitosan in acetic acid. After homogeneous mass was received, the mixture was spread on the mold surface to obtain the final thin layer of approx. 0.5-2 mm, and air-dried at room temperature. The above mentioned method has characteristics of novelty because it includes application of very specific concentrations of the components (chitosan, agarose), and solvents (acetic acid and sodium hydroxide), allowing for the neutralization of the acidic chitosan solution without its cross-linking followed by gelation.

Cell culture test. The study was conducted using human normal skin fibroblasts (BJ) obtained from ATCC. The cytotoxicity of the produced film was determined according to ISO 10993-5:2009 by indirect test using fluid extract of the chit/aga biomaterial. Cell viability was assessed upon exposure to the extract using MTT test. Biocompatibility of produced film was also investigated using LIVE/DEAD cell viability assay. In addition, fibroblast proliferation was assessed byWST-8 test and fluorescent staining of cytoskeleton and cell nuclei using phalloidin and DAPI dye, respectively.

Results and Discussion

Carried out research showed that developed material was non-toxic since the cytotoxicity MTT assay demonstrated that the viability of BJ cells exposed to the extract of chit/aga film was high and exceeded 101% comparing with control sample (TABLE 1). Moreover, confocal microscope observation presented spread and flattened cells on the surface of the produced film, indicating that fibroblasts were well attached (FIG. 1). The material was not only non-toxic, but also provided optimal conditions for cell divisions as WST-8 test revealed significant increase in cell number with time, which proves the favorable conditions prevailing on the top surface of the film (FIG. 2).

TABLE 1. Viability of fibroblast cells determined by MTT test upon exposure to the extracts of chit/aga film.

	Viability [% of control] ± SD
BJ 24h	98.32 ±0.02
BJ 48h	101.72 ± 0.031
Positive control	3.16 ± 0.003

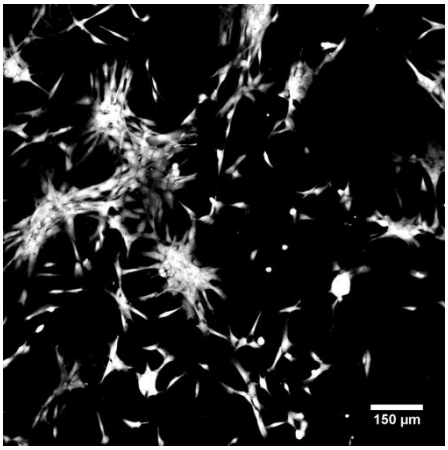

FIG. 1. Fibroblast cells growth on the chit/aga film assessed by LIVE/DEAD staining.

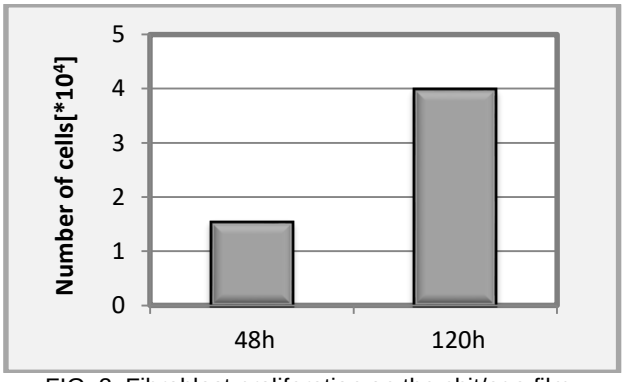

FIG. 2. Fibroblast proliferation on the chit/aga film assessed by WST-8 test.

Conclusions

Thin film produced by pH neutralization at the production stage, that does not lead to chitosan cross-linking and gelation, is characterized by non-toxicity, and also possesses surface which is supportive to cell adhesion and proliferation. Thus, chit/aga-based film can be potentially used in tissue engineering as skin substitute for acceleration of wound healing process.

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. The study was also supported by HQMedEd project of Medical University of Lublin, Poland.

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NOVEL AGAROSE/β-1,3-D-GLUCAN FOAM AS PROMISING BIOMATERIAL FOR SKIN REGENERATION APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 21]

Introduction

Skin wounds (especially chronic) often do not heal according to the expectations and provided treatment. In this case, it is necessary to use bioactive wound dressings. Currently, high hopes are connected with biomaterials based on β-glucans, which exhibit multifunctional properties, including moisturizing properties, antioxidant activity, anti-inflammatory and regenerative effects. Dressings made of β-glucans appear to be a suitable alternative in tissue engineering since they are very stable, flexible and resistant to proteases [1]. Recent reports indicated the possibility of using bacterial β-1,3-D-glucan (curdlan) in the production of biomaterials [2]. It is known that curdlan aqueous suspension create high-set thermal nonreversible gel at temperature > 80°C [3]. It can be also combined with other biocompatible polysaccharides (e.g. agarose, chitosan) to improve its biocompatibility, absorption ability, and ensure adequate gaseous exchanges. The main purpose of this work was to create a new agarose/curdlan matrix-based foam for regenerative medicine applications, including the treatment of exudative wounds.

Materials and Methods

Preparation of foam

Agarose/curdlan foam was prepared by mixing agarose and curdlan at the appropriate ratio to prepare suspension in deionized water. Obtained homogeneous mass was transferred to a mould, which was incubated in a water bath at 95°C for 20 min. Then, the biomaterial was cooled to 4-8°C, moved to -80°C for 1-2 hours, and lyophilized for 16 hours. The resulting foam material was subjected to further testing.

Cell culture test

The cell culture experiments were carried out using human normal skin fibroblasts (BJ) purchased from ATCC. To assess cytotoxicity of the produced foam, indirect test (MTT assay) using fluid extract of the agarose/curdlan biomaterial was conducted according to ISO 10993-5 (2009). Viability of cells growing next to the samples and on the biomaterial was investigated by fluorescent stained using Live/Dead Double Staining Kit following with manufacturer protocol. Stained cells were then evaluated qualitatively by observation under a confocal laser scanning microscope (Olympus Fluoview equipped with FV1000).

Results and Discussion

The conducted MTT test showed that the tested material is not-toxic to human skin fibroblasts. Compared to the control, the viability of cells was slightly reduced on the second day of the test to 94,31%. Visualization by confocal microscopy showed clusters of viable fibroblasts around the material and only single cells growing on the surface of the agarose/curdlan foam, indicating that developed biomaterial prevents adhesion of skin fibroblasts. It is desired feature when biomaterial act as a temporary dressing to cover the wound since it allows to remove the material after healing process without causing trauma to the wound bed.

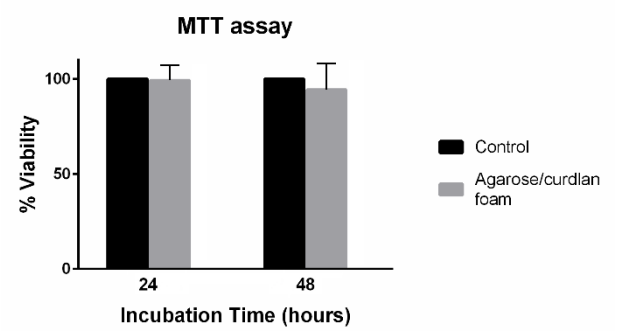

FIG. 1. MTT assay results for BJ cells cultured with fluid extract of the agarose/curdlan biomaterial.

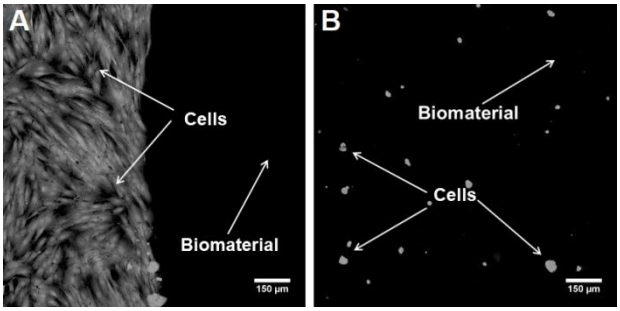

FIG. 2. Confocal microscope image of BJ cells growing next to the sample (A) and on the surface of the foam (B) after 2-day culture, magn. 100x, scale bar = 150 µm.

Conclusions

The obtained biomaterial is non-toxic and prevents the adhesion of skin fibroblasts to its surface. Due to specific surface properties of the material, it may be potentially used as a dressing, which when removed, does not affect the wound bed, preventing the scarring. Based on these studies, it can be assumed that the produced biomaterial have a promising potential to be used in skin regenerative medicine.

Acknowledgments

The study was supported by the 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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POLYMERIZATION SHRINKAGE AND COMPRESSIVE STRENGTH OF MICROHYBRID DENTAL COMPOSITE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 22]

Introduction

The UV light-activated composite resins are commonly used for hard teeth tissues restoration. The photopolymerization of such composites is accompanied by polymerization shrinkage which causes either marginal gaps or, in case of enduring adhesion, stress within the tooth or the restorative material. Polymeric shrinkage depends on many factors such as filler type and content, monomer system, polymerization characteristics, volume and cavity design, restorative procedure and light intensity used for photo activation [1-3]. The aim of the work was an investigation of the influence of direction of UV light irradiation on the polymerization shrinkage and compressive strength of micro hybrid dental composite.

Materials and Methods

Cylinder-shaped samples were made from UV light-activated micro hybrid composite Zmack comp (colour A2) produced by Zhermack. Composite is designed for anterior and posterior restorations. It is composed of dimethacrylate resin, triethyleneglycol dimethacrylate and the inorganic fillers (barium-aluminium-borosilicate glass, silicon dioxide, titanium oxide) with a weight content of 77%. The particles size of the fillers is from 0,04 to 5 μm. Samples were made in glass tubes with the internal diameter of 2 mm and the height of 6 mm. Material was applied in layers up to 3 mm and each layer was light-cured for 20 s with the use of Blue Luxcer (800-1000 mW/cm^2). Three variants of irradiation directions were used: (1) from the bottom and top, (2) form the right and left side, (3) combination of variants 1 and 2 (FIG. 1). For each curing variant at least 5 samples were made.

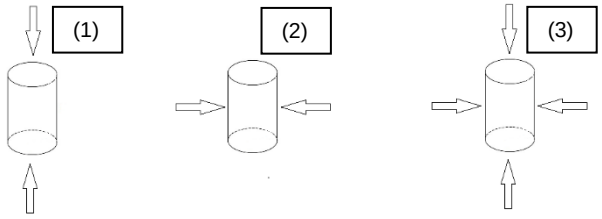

FIG. 1. Directions of specimens curing.

The polymerization shrinkage was calculated on the base of volume measurements according to the formula (1):

$$s = (1 - \frac{V_p}{V}) \cdot 100\% \qquad (1)$$

where: V_p is the volume of polymerized material and V is the volume of unpolymerized material.

The compression test was determined with the use of the MTS Insight 50 testing machine at a speed of 0,5 mm/min at a room temperature of 22±1°C. Registered force - displacement curves were recalculated into stress – strain curves and used for compressive strength (σ_c) and moduli (E_c) of elasticity calculations.

Results and Discussion

Tested composite belongs to the group of micro hybrid composites with high filler content which has an influence on polymerization shrinkage, since it leads, to a certain extent, to a reduction in monomer concentration. The shrinkage of the studied resin composites ranged from 1.17 to 2,35% depending on the direction of UV irradiation (TABLE 1). The highest value of volumetric shrinkage was for specimens irradiated from the bottom, top and two sides. Measured shrinkage of the tested composite is in the range of shrinkage of most dental materials with a range between 2 and 3% [4].

TABLE 1. The volumetric polymerization shrinkage.

	Irradiation directions		
	(1)	(2)	(3)
V [mm^3] ±SD	24,60 ±0,12	24,59 ±0,10	24,64 ±0,12
V_p [mm^3] ±SD	24,32 ±0,14	24,25 ±0,07	24,06 ±0,16
s [%] ±SD	1,17 ±0,13	1,36 ±0,18	2,35 ±0,32

Composites cured from the bottom and top (1) or form the right and left side (2) showed lower values of modulus of elasticity and compressive strength compared to composite cured from four sides (3) (TABLE 2). These results are correlated with the polymerization shrinkage and closer pacing of macromolecules for composite with higher shrinkage.

TABLE 2. The average values of maximum force (F_{max}), compressive strength (σ_c) and compressive modulus (E_c).

	Irradiation directions		
	(1)	(2)	(3)
F_{max} [N] ±SD	472,41 ±42,27	450,80 ±7,27	644,51 ±49,85
σ_c [MPa] ±SD	150,45 ±13,46	143,56 ±2,31	205,25 ±15,87
E_c [MPa] ±SD	7037 ±384	7034 ±580	9503 ±218

Conclusions

Dimensional stability of dental resin-composites is essential to the longevity and function of the restoration. Tested composite had quite high filler concentration. Higher filler content decreases the amount of resin in the composite, therefore polymerization shrinkage decreases.

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BACTERIOSTATIC LAYERS FORMED ON Ti-15Mo ALLOY SURFACE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 23]

Introduction

The process of implantation as a process interfering with the human body requires protection against bacteria attack (for example against *Staphylococcus* group of bacteria) that may cause the septic bone infection [1]. Due to the increasing resistance of bacteria to antibiotics, there is a necessity of developing research on alternative antibacterial agents increases [2]. An example of such an agent may be zinc [3], which has not only good efficacy against Gram-positive and Gram-negative bacteria but it also has biological properties that are beneficial to the human body [4]. In order to incorporate zinc into implant material, the plasma electrolytic oxidation (PEO) can be used. During this process the bioactive, porous oxide layer is formed [5,6].

Materials and Methods

Ti-15Mo alloy surface was treated by plasma electrolytic oxidation process in three different baths. All three of them consisted of 0.1 M $Ca(H_2PO_2)_2$ but different additives were used: ZnO, $Zn_3(PO_4)_2$ or the mixture of $Zn_3(PO_4)_2$ and $Ca_3(PO_4)_2$. During the PEO process the applied voltage was 150-300 V and the current density was 150 mA/cm^2. Using a scanning electron microscope equipped with energy dispersive X-Ray spectroscopy (Phenom Pro-X) the surface morphology, roughness and chemical composition of the oxide coatings was determined. The chemical composition was also determined by the XPS technique. The surface wettability was determined using water contact angle measurements (DataPhysics, OCA 15EC, Germany). In order to investigate the antibacterial properties of zinc containing Ti-15Mo oxide layers, the bacteria adhesion test was carried out using bacteria strains: reference *Staphylococcus aureus* (ATCC 25923), clinical *Staphylococcus aureus* (MRSA 1030), reference *Staphylococcus epidermidis* (ATCC 700296) and clinical *Staphylococcus epidermidis* (15560).

Results and Discussion

FIG. 1 presents the 3D SEM image of Ti-15Mo alloy surface after PEO process in bath containing $Ca(H_2PO_2)_2$ and ZnO. The pores and increased surface roughness visible on the images are the effect of PEO treatment. EDX analysis showed that the highest Ca/Ti and Ca/P ratio was obtained for the Ti-15Mo alloy surface anodised in condition of applied voltage 300 V and this value of voltage was chosen as the most optimal for the following tests. The wettability of Ti-15Mo alloy surface was measured and it changed after PEO process. The water contact angles decreased for surfaces anodised in all three investigated baths resulting in increasing the wettability of materials. The XPS survey spectra confirmed the presence of calcium, zinc, titanium, phosphorous and oxygen in PEO layer. The bacteria adhesion tests showed that all three types of investigated surfaces indicate bacteriostatic effect against *S. aureus* (ATCC 25923, MRSA 1030) and *S. epidermidis* (ATCC 700296, 15560). The amount of adhered bacteria decreased 10-100 times comparing to the reference bacteria concentration (~1·10^6 CFU/mL).

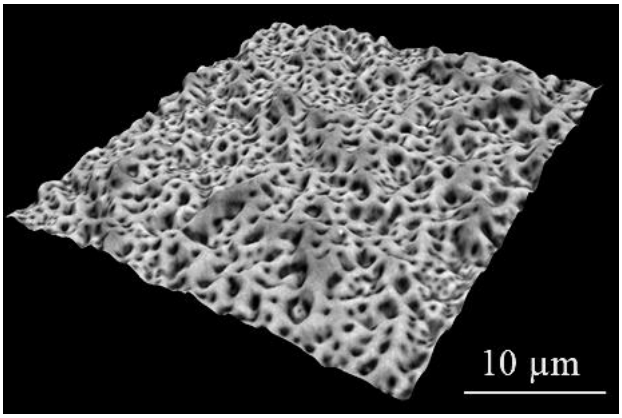

FIG. 1. 3D SEM image of Ti-15Mo surface after PEO (300 V, 150 mA/cm^2) treatment in 0.1 M $Ca(H_2PO_2)_2$ bath with an addition of 10 g/L ZnO.

Conclusions

Ti-15Mo alloy surface was modified by PEO in baths containing zinc compounds in order to obtain porous oxide layers with bioactive and bacteriostatic properties. As a result of PEO process, the roughness and wettability of Ti-15Mo surface increased. The presence of zinc in oxide layer was confirmed by the XPS technique. Obtained oxide layers with incorporated zinc indicated the bacteriostatic effect.

Acknowledgments

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OPTIMIZED MULTIPARAMETER CHARACTERIZATION OF STEM CELL-DERIVED EXTRACELLULAR VESICLES USING CLASSICAL AND IMAGING FLOW CYTOMETRY PLATFORMS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 24]

Introduction

The utilization of stem cells (SCs) combined with biopolymer scaffolds brings new hopes in a field of tissue regeneration. The unique properties of SCs involve not only their ability to directly differentiate in the site of injury, but also to release several paracrine factors, including extracellular vesicles (EVs), that are a heterogeneous group of small vesicular structures shed from the cell surface. Importantly, several data have demonstrated that EVs secreted by SCs populations cultured on biopolymer scaffolds may enclose bioactive content in the form of proteins and nucleic acids and transfer their cargo to the target cells. Thus, growing interest is placed on the utilization of those EVs in the field of biomedical research. However, there is still lack of standardized methods of EVs characterization which limits their applicability and affects data reproducibility. As an example, typical flow cytometry-based protocols, commonly used for cells phenotyping, may be inadequate for the characterization of EVs as particles with size close to the detection limit of conventional cytometers. Thus, the aim of this study was to optimize the use of two flow cytometry platforms for the multiparameter analysis of EVs isolated from different types of SCs populations.

Materials and Methods

EV samples were obtained from human iPS- and mesenchymal SCs (MSCs)- conditioned media by ultracentrifugation method. Next, high resolution flow cytometer Apogee A60 Micro-Plus dedicated to small particle applications was utilized to examine EVs phenotype, including expression of tetraspanins and surface markers. Furthermore, RNA Select dye was used to evaluate the content of RNA and the integrity of analyzed vesicles. Additionally, imaging flow cytometry platform (Image Stream Mk II) was also employed to visualize EVs on the single particle level.

Results and Discussion

Our results have revealed that two tested flow cytometry systems may be utilized for the phenotypic characterization of EVs secreted by human SCs populations. However, the conventional immunofluorescence staining and gating strategy protocols have to be thoroughly optimized. We have performed several controls and demonstrated the importance of additional preparation of reagent solutions prior to EVs staining. Additionally, the analysis of calibrating beads allowed to confirm the relative size of EVs and detection limits of tested cytometers. Importantly, utilization of Apogee and Image Stream systems enabled us to demonstrate the difference in the RNA content between iPS- and MSCs-derived EVs. Finally, we have also confirmed the presence of EVs subpopulations containing differential expression of exosomal markers, as well as surface markers characteristic for their parental cells.

Conclusions

In conclusion, we have demonstrated that the utilization of high-resolution flow cytometry platforms is a convenient method for the multiparameter characterization of EVs produced by different types of SCs populations, including those cultured on selected biocompatible polymer scaffolds.

Acknowledgments

This study was funded by NCBR grant STRATEGMED III (STRATEGMED3/303570/7/NCBR/2017) to EZS.

CELLULAR STUDIES ON PIEZOELECTRIC POLYVINYLIDENE FLUORIDE NANOFIBERS SUBJECTED TO ULTRASOUNDS STIMULATIONS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 25]

Introduction

In recent decades, there is an increasing interest in research related to development of the smart materials [1]. Such materials should response to external physical, chemical or mechanical stimuli and behave similar to natural body tissues. An example of smart materials are piezoelectric scaffolds, which can generate electrical signals in response to the applied stress [2]. Furthermore, they can stimulate the signalling pathways and thereby enhance the tissue regeneration at the impaired site. The piezoelectric scaffolds can act as sensitive mechanoelectrical transduction systems. It is known that electrical charges are crucial for various activity of cells. The major advantage of piezoelectric scaffolds is that electrical potential can be generated non-invasively under the influence of mechanical field, without the need of using invasive electrodes [3,4].

Materials and Methods

Polyvinylidene fluoride (PVDF, Mw = 400 000 g/mol) nanofibers were electrospun from 15% solution of dimethylformamide and acetone (DMF/Ac 4:1 weight ratio) at feed rate 0.2 mL/h (3 mm needle) and collected on drum collector (diameter 40 mm) at a distance between the needle and collector 180 mm:

- PVDF100 – collected at drum rotational speed 100 rpm and linear velocity 2 m/s resulted in random fiber distribution.
- PVDF1000 – collected at drum rotational speed 1000 rpm and linear velocity of 20 m/s resulted in aligned fiber distribution.

After the process, the samples were left for 24 hours for solvent evaporation.

Before *in vitro* studies, the samples were sterilized with UV light for 30 minutes. Fibroblasts L929 cells were cultured on the piezoelectric PVDF scaffolds.

Further, the samples with cultured cells were subjected to ultrasound stimulation for 30 minutes per day, for 7 days. Ultrasounds stimulus with power 20 mW, 80 mW and frequency 1.7 MHz were applied. As a control, piezoelectric PVDF scaffolds without ultrasonic stimulation were used (0 mW). In order to confirm the piezoelectric effect of the PVDF scaffolds on fibroblasts mitochondrial activity, MTT test was used.

The observations of fibers and cell morphology was conducted using Scanning Electron Microscopy (SEM).

Results were statistically analyzed using OriginPro v.8.

Results and Discussion

Scaffolds with random and aligned fibers orientation were produced.

Results of MTT test are presented in FIG. 1.

Mitochondrial activity of cells indicates the positive effect of piezoelectric phenomena on the cells under ultrasound stimulation.

This effect was similarly positive for low and high collector rotational speed. The observations using SEM verified the attachment and morphology of the cells.

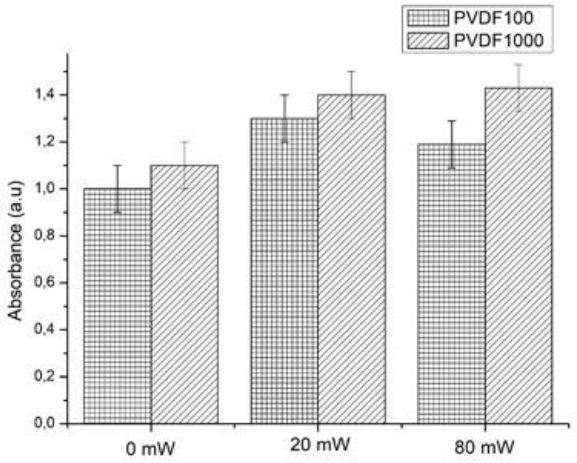

FIG. 1. Viability of fibroblasts L929 cultured on PVDF nonwovens and subjected to ultrasound stimulation (20 mW, 80 mW) after 7 day of cell culture.

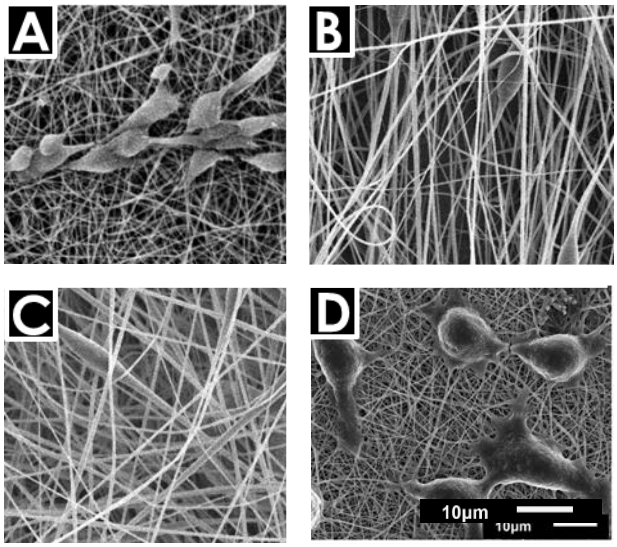

FIG. 2. SEM images of PVDF nanofibers: formed at different rotational speed of collector, 100 rpm (A) and 1000 rpm (B), with fibroblasts L929 (C, D) cell culture on day 7.

Conclusions

PVDF nonwovens as piezoelectric polymer stimulated by ultrasounds is advantageous for cells activity. The obtained preliminary results are promising from the perspective of tissue engineering applications.

Acknowledgments

Authors acknowledge Department of Ultrasounds PAS for sharing ultrasonic equipment for the tests.

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CHARACTERIZATION OF BICOMPONENT POLYCAPROLACTONE/GELATIN ELECTROSPUN NANOFIBRES CROSSLINKED WITH EDC/NHS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 26]

Introduction

Combination of synthetic and natural polymers for tissue engineering purposes has been proved to be advantageous for both designing optimal mechanical properties as well gaining bioactivity that widely used aliphatic polyesters lack [1, 2]. However, due to gelatin's proneness to dissolution in aqueous environment of human body, the benefits of its presence in bicomponent electrospun polycaprolactone/gelatin nanofibres could be short-lived [3]. Our approach to diminish this shortcoming is to crosslink gelatin within the fibre. We believe crosslinking with EDC/NHS does not cause any cytotoxicity and at the same time preserves material's properties.

Materials and Methods

A set of materials of different PCL to gelatin ratios were electrospun from solutions consisting of PCL (Mw = 80 kDa) and gelatin (300 bloom) dissolved in acetic and formic acid (9:1) mixture and in HFIP. PCL to gelatin ratios ranged from 9:1 to 1:1. EDC/NHS concentrations were set from 0,23%/0,12% to 0,02%/0,01%, with reaction times ranging from 1 to 9 hours. EDC and NHS were dissolved in ethanol and water in 7:3 w/w ratio. All samples underwent biodegradation studies for 1, 7 and 30 days in 37°C in PBS.
Samples were characterized by gelatin weight loss measurement, scanning electron microscopy, uniaxial tensile testing and cellular response studies.

Results and Discussion

Rapid gelatin loss was observed for all samples, the higher gelatin content the faster it progressed. Groove-like sites after gelatin leaching were clearly visible in SEM images of non-crosslinked samples after biodegradation test (FIG. 1b).
Judging both morphology, as well as mass loss of the samples, all chosen concentration/time crosslinking conditions were effectively preventing extensive gelatin depletion.

Conclusions

Excellent results in retaining fibres' morphology and gelatin content after 30 days of biodegradation can be achieved with crosslinking for just 1h using moderate EDC/NHS concentrations (FIG. 1c).
It was shown that EDC/NHS crosslinking method is a fast, effective and cheap way of preserving gelatin within the fibre and making sure the benefits of its use are lasting.

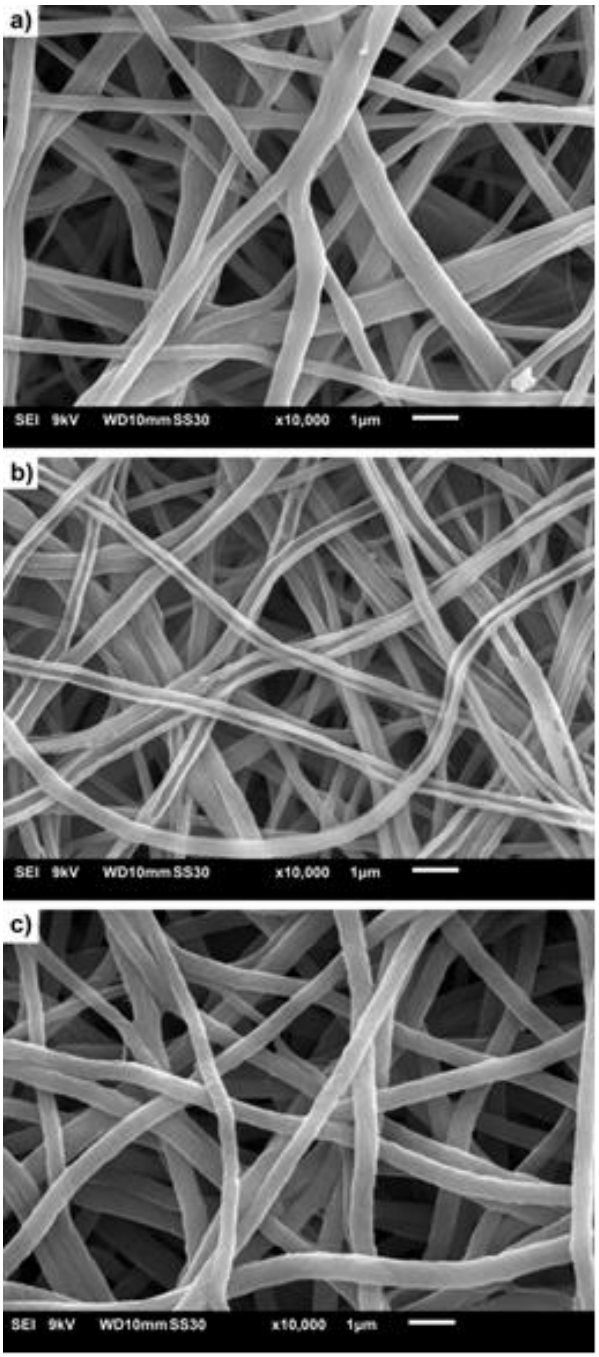

FIG. 1. Electrospun PCL/gelatin 7:3 material from acetic and formic acid mixture: a) not crosslinked, before biodegradation test; b) not crosslinked, after 30 days of biodegradation test; c) crosslinked, after 30 days of biodegradation test.

Acknowledgments

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BIOLOGICAL RESPONSE OF CH/Ag COATINGS DEPOSITED ON NiTi SHAPE MEMORY ALLOY

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[ENGINEERING OF BIOMATERIALS 153 (2019) 27]

Introduction

NiTi alloy due to the properties associated with the shape memory effects is increasingly used in medicine and veterinary medicine [1]. However, current applications require protection of the surface from unwanted migration of nickel ions. For this reason, the surface is modified with multifunctional layers/coatings, paying attention to the preservation of the shape properties of the NiTi substrate. One of the materials used for the coatings is chitosan and/or silver due to their bactericidal properties [2]. The paper attempts to evaluate the cell response to the silver content in the chitosan coating covering the surface of the NiTi alloy.

Materials and Methods

The alloy with a chemical composition of 50.6% at Ni and 49.4at% Ti was used as a substrate for the deposition of chitosan (Sigma Aldrich) coatings containing silver nanoparticles (AEE). The layers were electrophoretically deposited (EPD) using a deposition voltage of 20-40V and a deposition time of 30-120 s. The Ag content in the suspension was 0.6 g/l and 2 g/l. Cytotoxicity of the coated alloy was evaluated by MTT assay. Test was based on the activity of NADPH-dependent oxidoreductase enzymes which reduce the dye (MTT) to its insoluble form-formazan only in living cells. The cytotoxicity of coated alloy on L929 cells (NCTC clone 929: CCL 1) was assessed under the influence of eluates obtained from examined materials after 24 h of incubation (37°C, 5% CO_2) in culture media.

Results and Discussion

The deposition conditions (voltage and time) of the electrophoresis are determinants of layer properties composed of CH itself as well as the CH/Ag composite.
The first of all, increasing the deposition voltage results in increased adhesion of the chitosan layer to the surface of the NiTi alloy. Consecutively, the prolongation of the deposition time leads to an increase in the thickness and roughness of the layer. For example, by extending the time from 30 s to 600 s, the layer thickness can be increased from 0.5 μm to 12 μm [3]. In opposition to that, the surface roughness decreases as the deposition time decreases. An increase of the deposition voltage with a prolongation of the deposition time leads to an intensification of the electrophoresis process. In consequence, it causes an increase in the amount of emitted hydrogen. It blocks the flow of chitosan particles to the surface of the sample, strongly affecting the topography of the chitosan surface - the coating becomes rougher. The presence of silver in the suspension reduces the above effects and the surface, even with relatively long deposition times and high voltages (35 - 40V), adheres closely to the surface of the alloy.

Silver content in CH/Ag layer is strongly affected by concentration of silver in suspension, as well as by both values of deposition parameters. Increasing the amount of silver provides more material for building composite coatings. Hence, with the same voltage-time parameters, the amount of silver in the layer increases (FIG. 1). On the other hand, increasing the deposition voltage results in more intense silver incorporation in the chitosan layer. A smaller number of islands, formed by silver agglomerates, were visible on the surface. However, the agglomerates revealed a larger diameter [4].

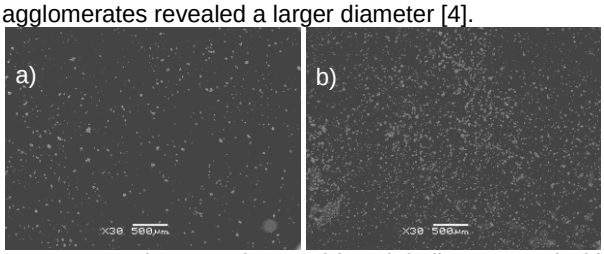

FIG. 1. SEM images observed for NiTi alloy covered with CH/Ag coating deposited at 25V/120s for Ag content 0,6 g/l (a) and 2 g/l (b).

Due to the content (0.6 g/l, 2 g/l) and the favorable distribution of nanosilver particles in chitosan, the alloys covered with 25V/120s and 35V/90s were selected for biological tests. The results of MTT assay on selected samples, NiTi alloy without cover and control group are shown in FIG. 2.

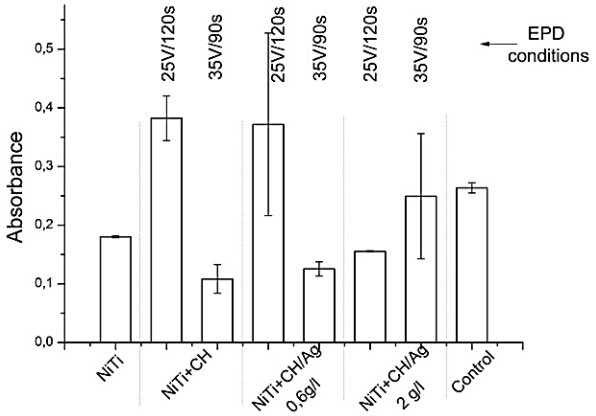

FIG. 2. Absorbance (λ = 570) of dissolved formazan (MTT assay) measured for studied groups.

Summary

The best results of cells proliferation were found in the alloy covered with chitosan as well as composite CH/Ag with a silver content in suspension of 0.6 g/l. This fact indicates that a small amount of silver does not adversely affect the proliferation of cells. By increasing the silver content in the CH/Ag coating (the amount of Ag up to 2 g/l and the deposition voltage to 35 V), cell survival drastically decreases by almost 70%. As a result, the cell survival rate is lower than for the uncoated alloy. This fact is related to both the thickness of the chitosan coating and the content of silver.

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BIOLOGICAL PROPERTIES OF ELASTOMERIC PHOTOCURED NETWORKS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 28]

Introduction

Over the past years, significant attention has been paid to biocompatible and bioresorbable polymer networks, which are the preferred replacements for individual stable biomaterials used in medical applications [1-2]. One of the modern methods of crosslinking is photopolymerization, through which liquid monomers turn into a highly cross-linked polymer in seconds. This technique is economical, does not require toxic solvents, high temperature or pressure, and allows precise control of the reaction by the light source and fast crosslinking rates. Mild reaction conditions enable preparing materials in situ, *in vivo* with potential applications in tissue engineering or implantology. Despite many advantages offered by photoinduced polymerization, there are several factors that can limit its effectiveness. One of them is oxygen inhibition, which leads to formation of a layer of non-crosslinked monomer on the material surface [3].
In this paper, we investigated the effect of the cross-linking atmosphere and the addition of a reactive cross-linker (tripropyleneglycol diacrylate (TPGDA)) on the properties of the elastomeric polymer networks obtained from a telechelic precursor comprising terminal methacrylic functionalities on a uretane-ester backbone. *In vitro* cell culture tests were performed to assess cell response to extracts from the tested materials. In order to investigate stability of the polymer network, hydrolytic and enzymatic degradation has been performed.

Materials and Methods

Telechelic precursors comprising terminal methacrylic functionalities and ester-urethane derivatives of dimer fatty acids were used to obtain elastomeric polymer networks according the procedure described in [2]. The chemical structure of the precursor was assessed by ATR-FTIR analysis, confirming the presence of ester and urethane bonds. Polymer networks were obtained *via* photopolymerization according to the following scheme (FIG. 1).

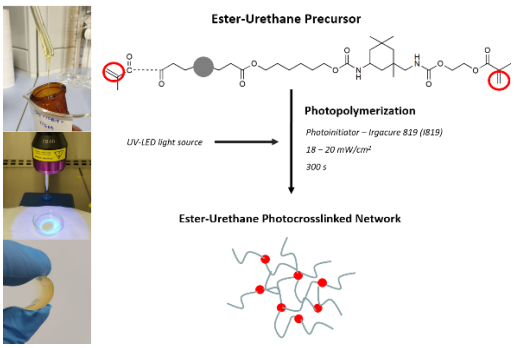

FIG. 1. Scheme of polymer networks preparation.

The crosslinking process was carried out in air or under argon. Cytotoxicity tests were performed on extracts of materials prepared with and without 25% wt. addition of TPGDA crosslinker, both in air and under argon. Polylactide (PLA, Resomer L210) and poly(ε-caprolactone) (PCL, CAPA 6430) were used as reference materials. Hydrolytic degradation of polymer networks (air atmosphere, 2% wt. I819, UVA 365 nm) was carried out in SBF solution at 37°C for 6 months.
Enzymatic degradation of polymer networks (argon, 25% wt. TPGDA, 1,5% I819, UVA 365 nm) was induced using lipase from *Pseudomonass cepacia* and carried out at 37°C for 42 days.

Results and Discussion

Cytotoxicity studies indicated that the crosslinking atmosphere and addition of reactive crosslinker (TPGDA) had a noticable effect on the viability of mouse fibroblast cells L929. Cells exposed to extracts of materials crosslinked under argon maintained a viability of ~90%, while extracts of same materials crosslinked in air were highly cytotoxic. (FIG. 2).

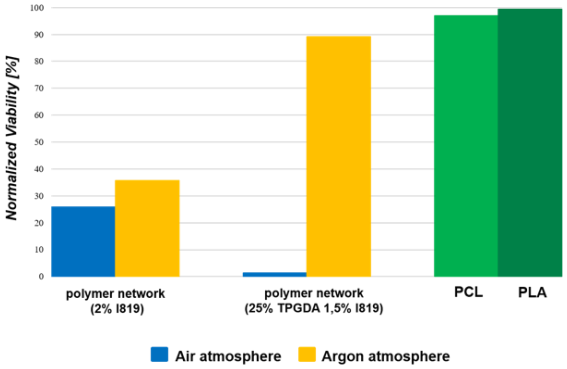

FIG. 2. Normalized viability of L929 cells in the presence of extracts from the tested materials.

The degradation studies, including mass loss measurements, showed that the materials are susceptible to both hydrolytic and enzymatic degradation. FTIR analysis identified changes in the functional groups present in the material structure. The most visible changes were observed in spectral features assigned to ester and urethane groups.

Conclusions

Ester-urethane polymer networks can be synthesized from telechelic macromonomers containing dimer fatty acids derivatives *via* photopolymerization. Cytotoxicity tests indicated that the combination of inert gas atmosphere present during crosslinking and the addition of a reactive crosslinker (TPGDA) yield a material that exhibits minimal cytotoxicity to L929 mouse fibroblast cells. Extracts from samples crosslinked in air exhibited relatively high cytotoxicity, likely due to oxygen inhibition, which results in a layer of unreacted monomer on the surface of the material and from which cytotoxic substances are release. The crosslinked materials were susceptible to hydrolytic and enzymatic degradation, as evidenced by registered changes in chemical structure and weight loss.

Acknowledgments

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MODIFICATION OF HYDROXYAPATITE BY ANIONIC SUBSTITUTION

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[Engineering of Biomaterials 153 (2019) 29]

Introduction

The healing of bone defects requires materials that enhance regeneration of bone tissue. Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is often used as filler of bone defects due to its biocompatibility, bioactivity and osteoconductivity. Application of HAP substituted with anions such as F^-, Cl^- or CO_3^{2-} instead of pure HAP could bring additional benefits such as enhanced cell proliferation and differentiation [1].

Fluorine is an essential trace element in bone tissue, which can promote the crystallization of calcium phosphate and accelerate the mineralization process [2]. However, a high concentration of fluoride was suggested to reduce osteoconductivity and also to cause adverse effects, such as osteomalacia. Therefore, it is necessary to control the release of fluoride ions into the environment.

Aim of the study was to determine and compare some biological and physicochemical properties of anion-substituted apatite and pure HAP such as cytotoxicity, ion reactivity and level of fluoride releasing from granules.

Materials and Methods

Preparation of HAP and FAP

HAP and fluoride-doped apatite (FAP) granules were synthesized using sol-gel method. Granules of a size 0,2-0,3 mm were used in the study. 800 °C was chosen for calcination treatment of apatite precursor.

Ion reactivity assessment

Before the experiment, FAP/HAP granules and SBF solution were prepared and sterilized. 0,5 g of every granules were soaked in 10 ml of SBF for 28 days at 37°C. Every 2-3 days fluid was exchanged and examined for calcium and phosphates concentration.

Fluoride ion release test

Fluoride release was determined in PBS solution for 15 days. 1 g of each type of granules were soaked in 10 ml of PBS and kept at 37°C. Fluoride content was measured in defined time points (0; 1; 2; 4; 8; 12; 24; 48; 72; 96; 120; 240; 360 hours) with the use of fluoride ion selective electrode.

Mercury intrusion porosimetry

Pore size distribution and other physical parameters of the granules were evaluated using Autopore IV 9500 (Micrometrics Inc) mercury porosimeter.

Cell culture experiment

Cytotoxicity of the biomaterials was evaluated indirectly by means of fluid extracts obtained by immersing the test materials in a complete culture medium supplemented with 2% FBS under standard conditions: 24 h, at 37ºC in a humidified atmosphere of 5% carbon dioxide and 95% air. After 24 h exposure to the extracts, viability of cells was determined using MTT test.

Results and Discussion

FTIR spectra show effect of fluoride substitution on hydroxyapatite structure (FIG. 1).

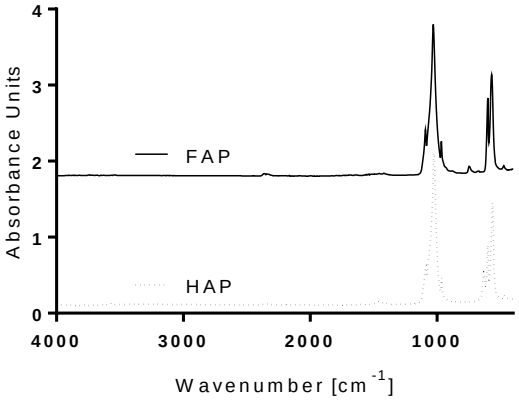

FIG. 1. FTIR spectra of hydroxyapatite and apatite modified by fluoride substitution.

Mercury porosimetry measurements revealed higher bulk density and lower porosity of FAP than HAP granules.

Ion reactivity assessment showed that both materials decreased concentration of Ca^{2+} and PO_4^{3-} in SBF solution, while HAP exhibited greater uptake of these ions than FAP.

Fluoride release profile from the FAP to PBS medium with a pH value of 7.4 revealed high release rate in the first 48 h of the experiment. Maximum concentration was 0,5 ppm.

Cell culture experiment proved the significant differences between cytotoxicity of FAP and HAP granules (FIG. 2). FAP extract did not cause significant changes in cell viability after 24 and 48 hours, while HAP reduced cell viability to the level 70-80% of control.

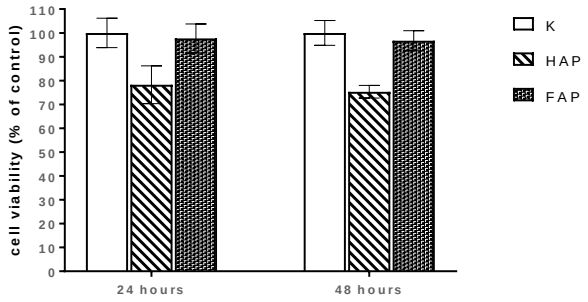

FIG. 2. Cytotoxicity evaluation of the granule extracts with the use of MTT test against human osteoblasts (hFOB 1.19).

Conclusions

In the present study, apatite modified by anionic substitution was synthesized and its properties were compared with pure hydroxyapatite. On the basis of obtained results, we suggest that fluoride-substituted apatite is a promising material for the use in bone tissue engineering.

Acknowledgments

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GRAPHENE OXIDE MODIFIED WITH COLLAGEN I FOR MYOCARDIAL TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 30]

Introduction

Nowadays a lot of scientific effort is focused on the development of new materials for the biomedical use (e.g. for tissue engineering [1] and cancer treatment). One of the most promising material in this field is graphene derivative called graphene oxide (GO). GO is a defected graphene (carbon layer with one atom thickness arranged in hexagonal crystal lattice), where defects are the result of reactive oxygen functional groups bonded to the surface. Great interest of GO is due to its physicochemical properties which allow for modifications of GO structure by different biomolecules attachment. This extend possibilities for interaction with different types of cells and tissues. Moreover, GO without any surface modification was found to be nontoxic and biocompatible towards different cell lines, even human mesenchymal stem cells. [2]

High-molecular weight proteins e.g. collagen are often introduced into biomaterials to improve cell attachment and proliferation. However, bonding such molecules of desired amount with material poses many difficulties. Collagen I fibrils (Col I) is a major compound of Extracellular Matrix (ECM). Furthermore, functionalization of GO with Collagen I contributes in to the mechanism of the cell differentiation from the Mesenchymal Stem Cells (MSC) to the Cardiomyocytes. [3]

The aim of this study was to obtain GO layers modified with Collagen I with the proper distribution of this peptide fibrils on the surface of GO flakes. Here we present several ways to prepare such composite with different GO coverage and morphology of Collagen I.

Materials and Methods

GO was obtained by modified Hummers method. GO flakes with the concentration of 1 mg/ml were deposited on the glass surface (10 μg/cm^2).

In the first experiment Col I dissolved in acetic acid was mixed with N-(3-dimethylaminopropyl)-N' ethylcarbodiimide) (EDC) and N-Hydroxysuccinimide (NHS), put on GO surface and left overnight to react. In the second experiment firstly functionalization of the graphene oxide by the solution consisting of EDC and NHS was conducted. After that Col I dissolved in acetic acid was put on such modified GO surface with EDC and NHS residues and left overnight. In the third experiment collagen I dissolved in acetic acid was put on modified GO surface (without EDC and NHS residues) and after 24 hours EDC and HNS solution was dropped on thin Col I layer (not dried) bonded to GO surface. It was left overnight again to react. Applied concentration of Collagen type I was 1 pg/ml, EDC and NHS were 20 mg/ml and 15 mg/ml, respectively.

Results and Discussion

In case of each type of biofunctionalizations successful bonding between the GO flakes and Col I fibrils was noticed. That was confirmed by FTIR measurements where characteristic amide groups (C=O stretching (Amide I), N–H bending (Amide II) and C–N stretching (Amide III) are present. Moreover, the clusters of the Collagen I were observed with the use of SEM. The presence of Collagen I clusters was noticed for each type of applied modifications.

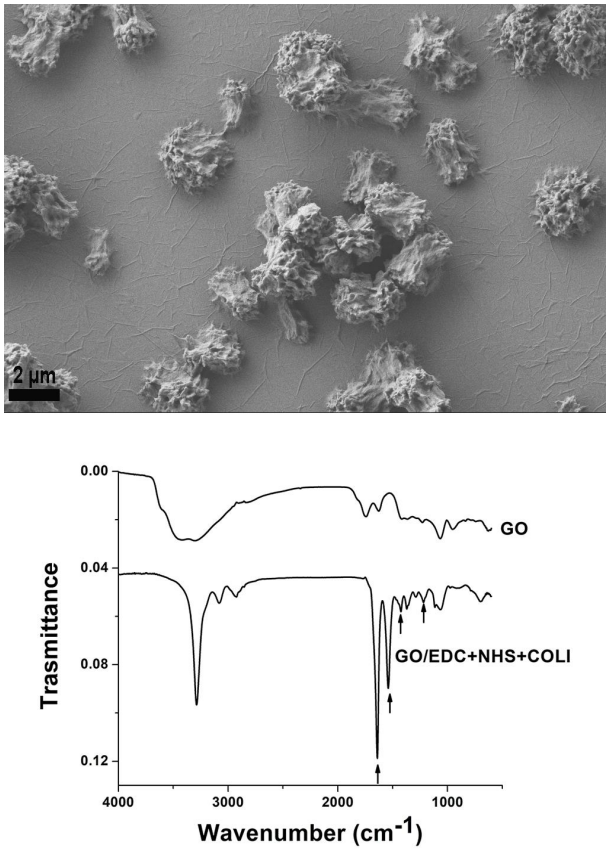

FIG. 1. SEM image and FTIR spectra of the GO modified with Col I mixed with EDC and NHS.

Conclusions

According to the obtained data, the optimal distribution of the Collagen I was received for the third experiment, where suspension of Collagen I was deposited on the modified graphene oxide and crosslinked by the addition of EDC/NHS solution at the last step. That method allowed to receive the homogenous distribution of Collagen I on the surface of graphene oxide flakes. Additionally, the presence of the empty spaces, where Collagen I was not bounded can allow for better interaction between the cells and graphene oxide flakes. Moreover, it has potential application for the additional immobilization of other molecules such as growth factors or drugs (drug delivery system).

Acknowledgments

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GOODBYE HOSPITALS AND HELLO IMPLANTABLE SENSORS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 31]

Introduction
There is an acute shortage of organs due to disease, trauma, congenital defects, and most importantly, age related maladies. While tissue engineering (and nanotechnology) has made great strides towards improving tissue growth, infection control has been largely forgotten. Critically, as a consequence, the Centers for Disease Control in the U.S. have predicted more deaths from antibiotic-resistant bacteria than all cancers combined by 2050, culminating into a prediction of 3 deaths every second. Moreover, there has been a lack of translation to real commercial products. This talk will summarize how nanotechnology with FDA approval can be used to increase tissue growth and decrease implant infection without using antibiotics. Studies will also be highlighted using nano sensors (while getting regulatory approval).

Methods
We have grown nanoparticles and induced nanoscale surface features on numerous implants inserted today. We have further grown sensors off of currently implanted biomaterials. Lastly, we have fabricated a wide range of self-assembled materials using them to both increase tissue growth and reduce infection. This talk will emphasize both in vitro and in vivo studies.

Results and Discussion
Our group has shown that nanofeatures, nano-modifications, nanoparticles, and most importantly, nanosensors can reduce bacterial growth without using antibiotics. This talk will summarize techniques and efforts to create nanosensors for a wide range of medical and tissue engineering applications, particularly those that have received FDA approval and are currently being implanted in humans. Moreover, our nanosensors can communicate to hand held devices cellular events at the surface of the implant and, in turn, such sensors can communicate back to release molecules that reduce infection, inhibit inflammation, and/or increase tissue growth.

Conclusions
Nanotechnology has proven to be a technology that can be approved by the FDA to improve tissue growth, limit infection, and inhibit inflammation without the use of drugs. Further nanosensors can be implanted with biomaterials to determine their fate and even control cellular events to promote success. In this manner, nanotechnology is revolutionizing healthcare.

NEW COMPOSITES BASED ON BACTERIAL NANOCELLULOSE AND COLLAGEN

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[ENGINEERING OF BIOMATERIALS 153 (2019) 32]

Introduction

Bacterial nanocellulose (BNC), also defined as microbial cellulose, is produced by bacteria. The valuable properties of BNC include, in particular, unique biological, physicochemical and mechanical properties. An example of exceptional properties are high crystallinity, high water-holding capacity, excellent tensile strength and also Young's modulus. BC is used in medicine as wound dressings, drug carriers, medical implants and in cosmetology. It also has a significant role in various industries, i.e. food, paper, textile, chemical industries. Collagen is widely used for production of 3D sponges, wound dressings and scaffolds for biomedical applications (1). It is also widely used in cosmetic preparations (2). Due to several requirements in biomaterials field there is a need to modify natural polymers for preparation of new materials and/or composites. Binary blends of two natural polymers can lead to preparation of new materials suitable for biomedical applications (1,3). In this work the composites based on bacterial nanocellulose and collagen were prepared and its properties were studied.

Materials and Methods

Bacterial nanocellulose was obtained from Center of Polymer System Tomas Bata University in Zlin, Czech Republic. Collagen (Coll) was obtained in our laboratory from tail tendons of young rats.
Bacterial nanocellulose was covered by collagen solution and after solvent evaporation the composite was obtained. For comparison also gelatin was used to prepare the composite. The structure of the composite was evaluated by attenuated total reflection infrared spectroscopy and Scanning Electron Microscope (SEM) pictures. Surface properties of thin films were analyzed by AFM and contact angle measurements.

Results and Discussion

After solvent evaporation from collagen and gelatin solutions poured onto a bacterial nanocellulose films the composites made from bacterial nanocellulose and collagen/gelatin were obtained. IR spectroscopy showed that between components of the composite there are interactions. According to the structure of single biopolymers the interactions are due to hydrogen bonds formed between chemical moieties of polymers. After solvent evaporation from the soluble polymer thin films were obtained, which covered the nanocellulose surface forming new composite.

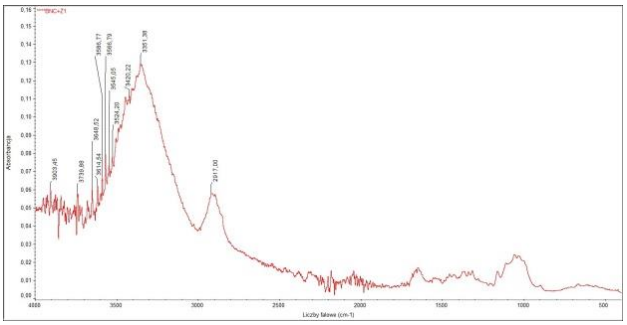

FIG. 1. IR spectra of BNC covered with gelatin.

Film-forming properties and good adhesion of collagen and gelatine lead to the formation of bilayer composites with BNC.

Conclusions

Strong interactions between bacterial nanocellulose and collagen and/or gelatin can lead to new composite material. The modification of material properties is a consequence of the strong interaction between the polymeric components and the chemical structure of single biopolymers. Biological properties of new materials should be studied. New material can be considered as wound dressing material.

Acknowledgments

Authors acknowledge Center of Polymer System Tomas Bata University in Zlin for preparation of bacterial nanocellulose for this study.

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THE INFLUENCE OF COLLAGEN/THYMOL MATERIALS ON DEHYDROGENASE ACTIVITY AND ATP LEVEL OF PATHOGENS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 33]

Introduction

The overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria. Antibiotic-resistant bacterial infections become one of the main worldwide health problems. Because of that, researchers are looking for new, more efficient solutions. Currently, plant-based substances are extensively tested. Thymol is one of the major phenolic compounds naturally occur in essential oil from *Thymus vulgaris* and *Origanum vulgare* [2,3]. Since the Food and Drug Administration recognized thymol as "generally safe", thymol become popular in medical, food and cosmetics field.

Materials and Methods

Materials based on collagen (Coll) with thymol (T) addition (0.25-4 mg thymol concentration) were prepared using the solvent casting method for better miscibility nonionic surfactant (NS) were added. Materials with different concentration of incorporated thymol were cut for 10 mm x 10 mm squares and sterilized using UV light. After sterilization, nutrient broth and cell culture (*Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*) were added to materials and incubated in 37ºC. After 90 min, part of the medium from each well was collected and transferred to sterile 96 well plates, where all next steps of analysis were performed. Dehydrogenase activity test was performed using CellTiter 96 AQ One Solution Cell Proliferation Assay. Absorbance was measured at 490 nm (GloMax Discover, Promega).

ATP level was analyzed using a luminescence BacTiter-Glo™ Microbial Cell Viability Assay. All steps were performed according to manufacturer guides.

Results and Discussion

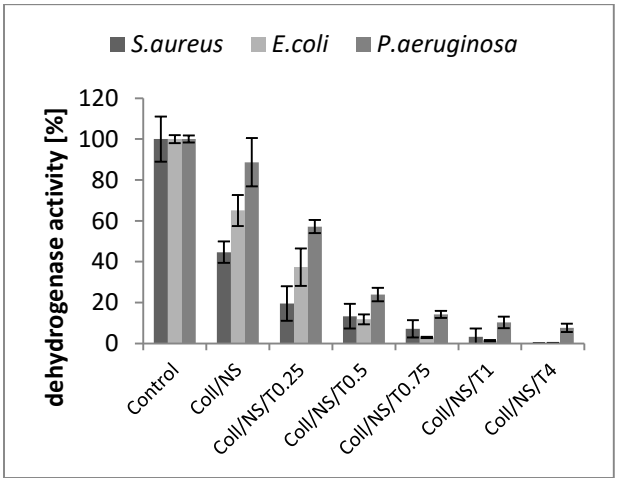

FIG. 1. Dehydrogenase activity of *S. aureus*, *E. coli* and *P. aeruginosa* after contact time with materials.

S. aureus, E. coli, and *P. aeruginosa* were chosen for this study. *S. aureus* is the most common bacteria involved in the initial stage of the infectious process [4]. *E. coli* and *P. aeruginosa* were found in chronic wounds [4]. dehydrogenase activity was inhibited between 11-55% and depends on bacteria strains. Thymol presented into materials, decrease the dehydrogenase activity. The most sensitive for thymol action were *S. aureus* and *E.coli*. Thymol addition in a concentration of 4 mg inhibited all dehydrogenase activity in those strains.

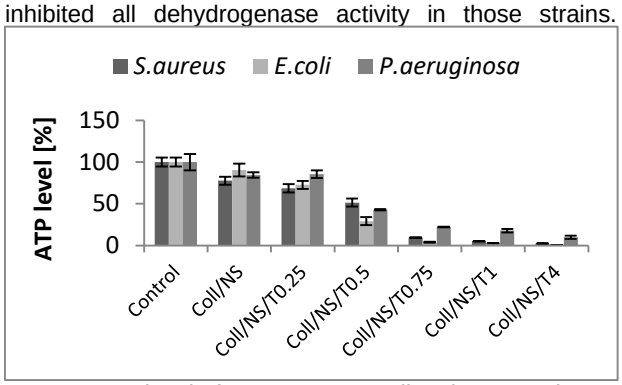

FIG. 2. ATP level of *S. aureus*, *E. coli* and *P. aeruginosa* cells after contact time with materials.

The adenosine triphosphate (ATP) is only produced into cells within phases of metabolic activity, it is not stored and degrades after cell death [5]. The ATP level measurements were collected in the form of relative light units (RLU). During this study, ATP standard curve was not utilized, therefore the ATP luminescent signal (RLU) could not be converted into units of ATP. However, the luminescent signal could still be used as an indication of viable cells being presented. The results are presented as a percentage value of ATP level treating positive control sample as 100%.

ATP is universal energy molecule and cells may generate ATP during oxidative phosphorylation or substrate level phosphorylation [6]. Therefore, dehydrogenase activity and ATP level are related. In our study, the relationship between thymol amount, dehydrogenase and ATP level was similar.

Conclusions

Thymol and some other phenolic compounds, which occurs in essential oils, are known with their ability to interacting with bacterial cells membrane by hydrogen bonds. Resulting in the disruption of cells membrane and causing the leakage of cellular components. Therefore, the inhibition of dehydrogenase activity and ATP level may be related to thymol mechanisms of antimicrobial action.

Acknowledgments

Financial support from the National Science Centre (NCN, Poland) Grant No 2018/31/N/ST8/01509 is gratefully acknowledged.

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THE IN VITRO ANALYSIS OF THIN FILMS BASED ON CHITOSAN/TANNIC ACID

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[ENGINEERING OF BIOMATERIALS 153 (2019) 34]

Introduction

The surface roughness affects cells response immediately after the material implantation. Moreover, a rough surface inhibits the biofilm formation, the one of the main problems in implantation surgery [1]. Cell adhesion represents a molecular interplay between cell surface and the extracellular environment [2]. The material properties that affect cell adhesion will also influence cell division and they may either stimulate or inhibit cell growth. Thus, examining cell growth on the material with known surface parameters is the key first step to evaluate clinical potential of experimental biomaterials.
The aim of the study was to examine normal and cancer cells growth on the materials obtained by combining chitosan and tannic acid at 80/20, 50/50 and 20/80 ratios. The cell lines used in this study were the following: MNT-1 (human highly pigmented melanoma), SK-MEL-28 (human malignant amelanotic melanoma), Saos-2 (human osteosarcoma), HaCaT (spontaneously transformed aneuploid immortal keratinocyte cells) and human bone marrow-derived stromal cells (BMSC) obtained from a 56-year-old male patient (Institutional Review Board protocol nr 1072.6120.254.2017).

Materials and Methods

Tannic acid (M=1701.2 g/mol, TA) and chitosan (DD%=78, 1.8 × 10^6, CTS) are commercial compounds purchased from the Sigma-Aldrich Company (St. Louis, MO, USA).

Sample preparation

Chitosan and tannic acid were dissolved in 0.1M acetic acid, separately, at a concentration of 2%. The mixtures of chitosan and tannic acid were prepared in the weight ratios of 80/20, 50/50, 20/80. Mixtures were placed on a plastic holder for solvent evaporation. The thickness of the obtained films was 0.035 ± 0.003 mm. Results were compared with pure chitosan-based films as control.

Establishing cell cultures on the experimental films

All cells used in this study were seeded directly onto material films or tissue culture plastic (control TCP, Nest) at a density of 1x10^4/cm^2 in 1 ml of adequate serum-containing medium (SCM, TABLE 1). SCM was exchanged on day 2. On day 6 MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) was carried out in order to determine the metabolic activity of living cells. Briefly, at the day of MTS assay, cells were rinsed once with PBS (BioShop), supplemented with phenol-free Alpha-MEM (Gibco) containing 10 times diluted MTS reagent in the amount of 200 µl per well, followed by incubation in a CO_2 incubator. The reactions were developed until an apparent color change of the MTS reagent in culture wells vs. MTS reagent in empty (cell-free) well. Afterward, the MTS solutions from culture wells were transferred to individual wells in 96-well plates (Nest) and absorbance was measured at 492 nm using a plate reader (SpectraMax iD3 Molecular Devices). The intensity of the developed color is directly proportional to the amount of metabolically active cells, according to the technical bulletin of CellTiter 96® Aqueous One Solution Cell Proliferation Assay by Promega.

Results and Discussion

Metabolic activity of cells cultured on material films

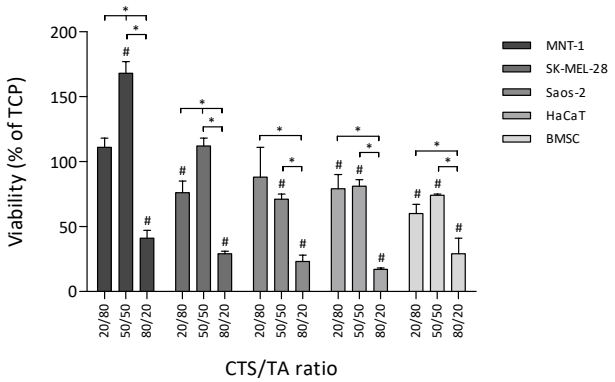

FIG. 1. Metabolic activity of different cell types cultured for 6 days on different material films. Results are displayed as mean ± STD (i.e. % change of cell viability on the material surface vs. cell viability on TCP) for cell lines MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. CTS/TA films combined in ratios 20/80, 50/50 and 80/20 were examined. #statistically significant vs TCP, *statistically significant between different material surfaces within particular groups.

Conclusions

Materials based on chitosan and tannic acid showed different influence on the normal and cancer cells. Films with the lowest tannic acid (CTS/TA 80/20) content inhibit the cell growth. The highest influence was noticed for MNT-1 cells and the lowest for BMSC. It can be observed that those films have higher surface roughness compared to other CTS/TA ratios. Thereby, it can be assumed that materials composed of chitosan and tannic acid may potentially find application in the cancer cells treatment. However, further experiments have to be carried out with expanded biological studies.

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COMPOSITE & HYBRID (BIO)MATERIALS BASED ON BIOACTIVE GLASS: TOWARD OPTIMISED SUBSTITUTE FOR BONE BIO-ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 35]

Introduction

From the need of support -as screws or plates (devices that must be easily shaped in 3D and that exhibiting specific mechanical properties)-, to the used of "gel-based device" -fillable or conformable, cellularized and/or cellularizable biomaterials (that exhibit highly hydrated properties with specific architecture in volume)-, bone regeneration necessitates various strategies and solutions to face complex, singular and multiparametric situations. Beside these specific requirements, bone substitute materials must present “osteo-properties”: from osteocompatibility to osteogenic and osteocompetency. In this context, due to its ability to release ions in solution and triggering signaling pathways that lead to osteoresponse [1], bioactive glass (BG) appear as a versatile, pertinent and universal solution whatever the bone material and the bone strategy developed. Moreover, its composition can be tuned in order to tailor its dissolution properties and/or to favor release of therapeutic ions.

Here we present the development of two different BG-systems: i) a composite based on PLA and ii) an hybrid based on gelatin (where the covalent linking between the organic and inorganic phases is insured by the (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) [2]. For each system, two BG particles composition are used, 13-93 and 13-93B20 (13-93 with 20% of the SiO_2 replaced with B_2O_3).

Materials and Methods

Each system contains 70% of organic matrix (PLA or gelatin) and 30% of BG (13-93 or 13-93B20 particles weight %). The composites were processed by co-extrusion whereas the hybrids were synthetized by sol-gel transition. First, the gelatin is functionalized with the GPTMS and then the BG is added in the solution. Different proportions of GPTMS/gelatin (molar ratio) were tested in order to vary the degree of connectivity between the organic and inorganic phases.

The dissolution of both composites and hybrids materials was studied in Simulated Body Fluid (SBF) up to two weeks. The change in the SBF pH was studied and correlated to ion release, measured by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM-EDX) confirmed the bioactivity related to the precipitation of an apatite layer at the materials’ surface when immersed in aqueous solution. Mass loss of the hybrids was measured and correlated to the degree of cross-linking between the gelatin and the BG particles.

Cell assays were performed in order to assess the osteoinductive potential of the materials. Osteopontin and myosin immunostainings as well as the alizarin staining were done after 14 days of incubation.

Results and Discussion

For the composites, over two weeks of immersion in SBF, the Si content in the solution increases due to the BG dissolution. The [P] and [Ca] concentration decreases overtime, suggesting the precipitation of a reactive layer. The reactive layer was further confirmed to be apatite. Precipitation of apatite was greater for the composites with boron containing glass, due to its faster dissolution rate. Myoblastic cells culture on the composites exhibits a decrease of myosin and increase of osteopontin. This indicates that the myoblastic cells in presence of BG go toward an osteoblastic lineage. Moreover, the neosynthese of a mineral matrix is evident (FIG. 1).

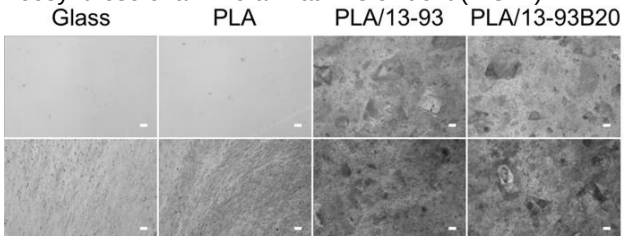

FIG. 1. Mineralization of C2C12 cells in DMEM studied with Alizarin red S staining after 14 days of incubation on glass, PLA, PLA/13-93, PLA/13-93B20 without cells (top line) or with cells (bottom line). Scale bare 200 µm.

For the hybrids, compare to a reference without bioactive glass and/or crosslinking, the mass loss is significantly lowered. This confirms the covalent linkages between the gelatin and the BG and the benefit in terms of stability. Moreover, higher is the ratio of GPTMS/gelatin, higher is the gels stability. *In vitro* dissolution of the scaffolds, performed in SBF, showed again precipitation of an apatite like-layer from BG dissolution (FIG. 2).

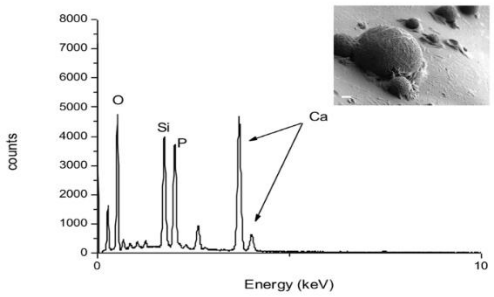

FIG. 2. EDX analysis of the nodules appearing on hybrid surfaces after two weeks of immersion in SBF.

Conclusions

These results demonstrate that the bioactivity of the BG was maintained in both composites and hybrids. Moreover, boron inclusion in the formulation allows a smart tailoring of the dissolution rate of the BG. Osteoinductive potential shown permits to conclude that such materials are promising for bone application.

Acknowledgments

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MULTIFUNCTIONALIZATION OF INERT CERAMIC SURFACES USING IN SITU CAP NUCLEATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 36]

Introduction

Bioinert ceramics such as alumina or zirconia have been commonly used in the field of orthopedics and dentistry due to its excellent mechanical properties, esthetic look, good biocompatibility and chemical inertness in biological environment. Activation of its bioinert surface could bring additional advantages for better implant-integration with surrounding tissues *in vivo*. Therefore, the aim of the present study was to develop an innovative biomimetic co-precipitation technique by using modified Simulated Body Fluid (SBF) to obtain a composite coating made of organic and non-organic components enhancing a bioactivation/functionalization of this inert biomaterial.

Materials and Methods

Zirconia samples were biomimetically coated by immersion in double-concentrated SBF-solution prepared according to Tanahashi *et al.* and kept at a body temperature for 3d [1].
Bovine Serum Albumin (BSA) has been chosen in 5 different concentrations (0.01, 0.1, 1, 10, 100 gL^{-1} and 0 gL^{-1} as a control) as a standard protein to be incorporated into the CaP-coating during the precipitation process. The incorporation of BSA into the SBF solution occurred on the half of the samples directly ("direct" coating) and for the other half on samples already pre-coated with SBF ("with pre-coating").
BSA/Alexa Fluor TM 488 conjugates were applied to visualize the incorporated proteins into the surface. To evaluate a role of sedimentation of protein in the solution, the coating produced on horizontal and vertical samples were compared. Samples were imaged by using fluorescence microscope. To determine the morphological changes on the substrate surfaces after soaking in SBF, scanning electron microscopy was applied. Carbon-content of the HAp-coating dependent on concentration of BSA in the solution were established by using EDX measurements. Moreover, the thickness of HAp-coatings could be measured by imaging of cross-section of ZrO_2-substrates.

Results and Discussion

The control samples (0 gL^{-1} BSA) as well as samples coated in SBF-solution containing 0.01 gL^{-1} BSA exhibit typical coral-like crystal structures [2] with app. 100 nm long crystal-plates. In contrast, with BSA-concentrations >0.1 gL^{-1} the crystal structure appears to be altered or protein-overlayed (FIG. 1).
The incorporation of protein within the HAp-coatings was visualized by using fluorescence microscopy to detect BSA/Alexa-FluorTM-488 conjugates, which gives a green signal. The intensity of green signal is stronger with increasing protein concentration in the solution. Additionally, the content of carbon was measured by EDX. The results show a logarithmic growth of carbon content in the HAp-coating with increasing BSA concentration in SBF solution by the precipitation process (FIG. 2).
The influence of the sedimentation process on the intensity of fluorescence signals proportional to the amount of proteins in the coating could also be observed. The results were in correlation with the chemical analysis of the coated surfaces (EDX).
Analysis of the cross-section of the obtained coating on CaP-pre-coated samples showed the apatite growth for all tested samples in comparison to the pre-coated control sample. The thickness of the coating decreases with the increase of protein concentration in the solution, which is in correlation with the SEM images.

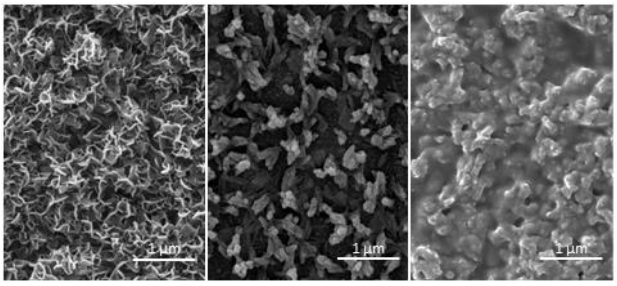

FIG. 1. Morphology changes through different BSA concentrations.

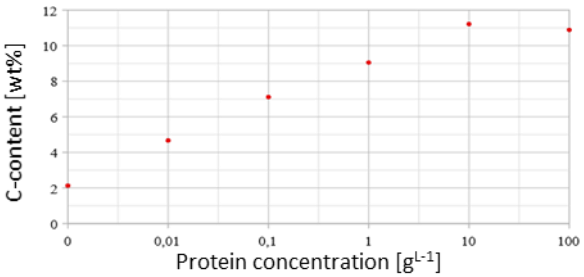

FIG. 2. Measurement of Carbon content by EDX.

Conclusions

It could be shown, that it is possible to co-precipitate an organic/non-organic coating based on HAp and biological agents such as BSA. This method could create a new biomaterial group, which surfaces could be tailored designed according to its desires and requirements. Based on these results with a standard protein, BSA has been replaced by specific proteins like Bone Morphogenetic Protein 2 (BMP-2) as a potential osteoinductive factor and Hepatocyte Growth Factor (HGF) as a growth factor. These proteins have already evidenced a strong influence on the crystal growth and the HAp-coating morphology as well. Further systematic analyses and cell culture tests are still on going in order to better understand biologically efficacy or bone growth factor response of the protein incorporated into the CaP-coating.

Acknowledgments

Funded by the Excellence Initiative of the German federal and state governments (Grant Nr. OPSF408).

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IN VITRO INVESTIGATION OF THE PLATELET AND LEUKOCYTE ACTIVATION ON DIFFERENT CRYSTALLOGRAPHIC SURFACES OF SILICON CARBIDE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 37]

Introduction

We aimed to reduce anticoagulant usage of patients against the thrombotic effects of artificial cardiovascular implants. The implants made of superior materials, which bring hemocompatible and mechanical properties together, can be tailored to achieve this goal. A promising candidate would be a high-strength ceramic with mechanical reliability, chemical inertness and biocompatible surfaces. In this research, cytocompatibility and hemocompatibility of a silicon carbide (SiC) single crystal were investigated in terms of crystallographic structure and surface atomic arrangement. Different crystallographic structures (single- and poly-crystal), surface terminations (Si-rich and C-rich) and polymorphs (4H and 6H) of SiC were used in order to reveal the interactions between blood content and material surface. The experiments were elaborately designed to control all parameters affecting the platelet activation and endothelial cell proliferation.

Materials and Methods

Advance diffractometer was used for the crystallographic phase composition analysis of ceramic specimens; the diffracted intensity versus 2θ is recorded by a detector. Surface roughness values of the single crystals were measured by atomic force microscopy (AFM). In order to gain a deeper insight on wettability, the static and dynamic contact angles of the samples were measured with an optical contact angle measurement and a contour analysis system.

Cytotoxicity of the specimens were analyzed by the live-dead staining assays for human umbilical vein endothelial cells (HUVECs) and blood cells. In order to examine the blood cell activation in terms of platelets and white blood cells, human peripheral blood mononuclear cells (PBMC) were isolated from the whole blood. Cell behaviors on the single crystal ceramics were analyzed under the dynamic flow conditions besides of the static cell culture conditions, in order to simulate physiological blood flow conditions of blood vessels. CD62P marker was used to investigate activated and adhered platelets by using ELISA assay. Visualization of the structure and interaction of blood cells with ceramic specimens were performed by using scanning electron microscopy (SEM).

Results and Discussion

AFM measurements verified that both monocrystalline and polycrystalline ceramic samples have comparable average surface roughness varying between 2 and 2.7 nm. According to contact angle measurements, all samples were found to be hydrophilic. However, there are differences in the degree of wettability. Cell culture results showed that the platelet activation and fibrin formation on single crystalline SiC are significantly lower than its polycrystalline form (FIG. 1). This can be attributed to the similar semiconducting properties of single crystalline SiC surface and blood proteins which lowers the surface charge transfer [1]. However, the surface charge transfer is not properly limited to prevent complete cell activation as in pure hemocompatible endothelial surface.

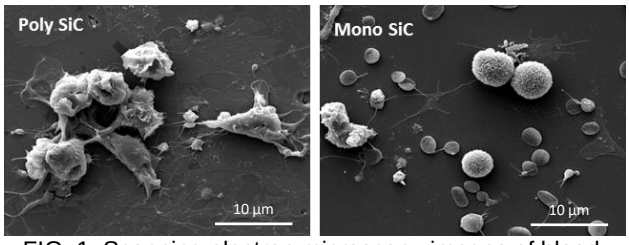

FIG. 1. Scanning electron microscopy images of blood cells on the single crystalline SiC (left) and polycrystalline silicon carbide (right) surfaces.

We also observed that Si-rich surface triggers the platelet activation and protein adsorption reproducibly and slightly more than C-rich surface, while 4H and 6H polymorphs showed no significant difference (FIG. 2).

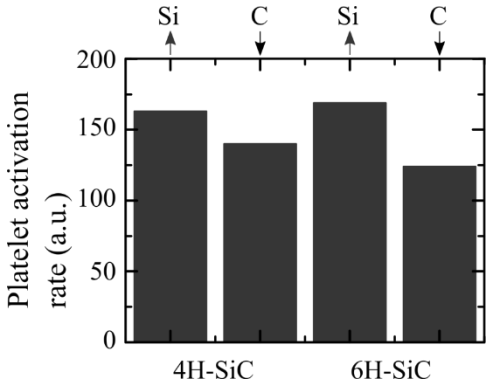

FIG. 2. Platelet activation rate depending on different crystallographic orientations and surface terminations of single crystalline silicon carbide.

Conclusions

The aim of the present work was to find out the influencing factors on the hemocompatibility. Polycrystalline and singe crystalline silicon carbide were examined by means of HUVECs and blood cells in order to investigate and compare their blood compatibility and cytocompatibility as a result of biological, morphological and physical characterizations. We conclude that, different crystal structures and surface terminations of silicon carbide play an important role on the cell responses. These findings provide vital information for the improvement of ideal surfaces for cardiovascular applications.

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SHORT CALCIUM PHOSPHATE WHISKERS AS REINFORCEMENT OF POLYMER COMPOSITES FOR BONE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 38]

Introduction

The necessity of replacing autogenous grafts with synthetic implants forces the development of new, improved materials that meet different requirements depending on the given need [1].

The worst bone injuries include heavy fractures as well as large bone defects caused by infections or cancers. In the case of the former, the promising biomaterials supporting regeneration may be solid composites for implants stabilizing the fractures (plates, screws, nails), characterized by very high mechanical strength, stiffness and degradability. However, injuries associated with the occurrence of bone defects require the use of filling materials that, apart from biocompatibility, resorbability and strength, will have an appropriate microstructure and optimal pore size that will allow good cell penetration, ingrowth of tissue, rapid vascularization and ease of nutrient delivery [2].

Meeting of such diverse requirements is expected primarily from polymer-ceramic composite materials. A well-known method of strengthening of these composites is the use of fillers with various morphology, especially thin fibers (whiskers). Apatite whiskers that exhibit biotolerance in the tissue environment, controlled resorption and the possibility of creating a permanent and strong connection to the bone are particularly widely considered. The whisker reinforcement effect can be explained by a shear lag analysis in which the whiskers can be loaded up to their fracture strength [3].

The aim of the work was verifying if the short Ca-P whiskers are proper to reinforcing porous and solid polymer composites. In the work we checked how the addition of short Ca-P whiskers affects the strength properties of porous composites and we compared the results with previously obtained results for solid composites [4]. As the polymer matrix in the composites a biodegradable polylactide was used which is widely used in orthopedic surgery as well as in tissue engineering scaffolds. The work shows also the research results on the effect of the whiskers on morphology, density and porosity of composites.

Materials and Methods

Ca-P whiskers used in this work were prepared in accordance with our previous work [5] and modified with lauric acid or γ-aminopropyltriethoxysilane (Sigma-Aldrich) to improve surface chemical compatibility between whiskers and the polymer matrix.

Solid composites were prepared by hot-pressing of casted and evaporated suspensions of Ca-P whiskers in solution of polylactide (Evonik) in dichloromethane (Avantor). The composites with 10 wt.%, 20 wt.% or 30 wt.% of Ca-P whiskers were prepared.

Porous composites were prepared by lyophilization of frozen suspensions of Ca-P whiskers in solution of polylactide in 1,4-dioksane (Avantor). The whiskers content in composites was in the range of 10-30 wt.%.

The morphology of whiskers and composites was evaluated by scanning electron microscopy (SEM). A Fourier transform infrared spectrometer was used to identifying the chemical functional groups. Density and porosity were determined by the liquid displacement method. Mechanical properties of the composites were evaluated by tensile or compressive tests.

Results and Discussion

The obtained results indicate that with the addition of whiskers to composites, the compressive strength of the porous composites may increase significantly (from 0,13 MPa up to 0,26 MPa for composites based on 4% polylactide solutions), while the tensile strength of the solid composites slightly decreases. The composites based on modified whiskers show higher compressive and tensile strengths than the composites with unmodified whiskers.

SEM observations show that short Ca-P whiskers are embedded in thin walls of the porous scaffolds providing them with reinforcement. The modified whiskers are better covered with the polymer than the unmodified whiskers and they're distributed more homogenously in the polymer matrix. That confirms better surface chemical compatibility between modified whiskers and the polymer matrix (FIG. 1).

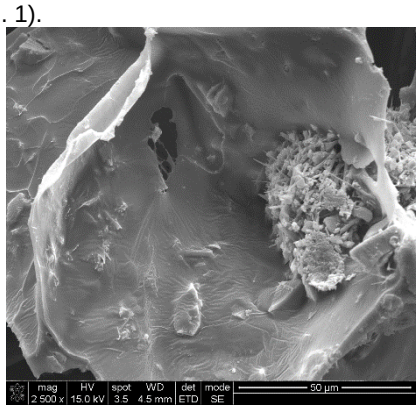

FIG. 1. SEM image of porous composite with the addition of modified Ca-P whiskers.

By manipulating of solutions concentrations and the content of whiskers, porous scaffolds with different densities and a wide pore size range (2-360µm) can be obtained – suitable for the growth of new bone tissue.

Conclusions

The addition of Ca-P whiskers affects the mechanical strength and morphology of as well porous composites as solid composites. The whiskers are proper to reinforcing the composites. The surface modification of whiskers allows for a better interfacial connection between these whiskers and the polymer matrix.

Acknowledgments

The work was financed from resources assigned to the statutory activity of the Łukasiewicz Research Network - Institute of Ceramics and Building Materials in Warsaw.

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MULTIFUNCTIONALIZATION OF INERT CERAMICS THROUGH GRAPHENE-DERIVATES IMMOBILIZATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 39]

Introduction
Bioinert ceramics, such as zirconia, provides the mechanical strength required in implants, but its limited bioactivity renders it incapable of osseointegration [1]. Thus, its biomedical application is limited [2]. Graphene and its derivatives exhibit excellent bioactivity and can enhance osseointegration, hemocompatibility and antibacterial properties [3]. Moreover, enzymes and proteins have been immobilized to graphene-derivates applying the reaction between the amine groups of proteins/enzymes and the carboxylic groups of GO/rGO to support biological properties or add another functions to graphene-derivates. Through immobilization of graphene-derivates to the ceramic inert surface, properties of rGO and ceramics could be merged to create a versatile biomaterial.
In the present study we introduce an innovative technique for functionalizing bioinert ceramic by immobilizing various graphene derivatives onto the surface by tailored self-assembled monolayer technique (SAM).

Materials and Methods
Several well-established and characterized graphene-derivatives exhibiting different morphologies, shapes and physico-chemical properties provided from Institute of Electronic Materials Technology, Warsaw (Prof. Lipińska), were selected to immobilize them on the ceramic surfaces (FIG. 1). Each graphene-derivative before coupling was biologically evaluated by using live/dead staining to ensure its cytocompatible character.

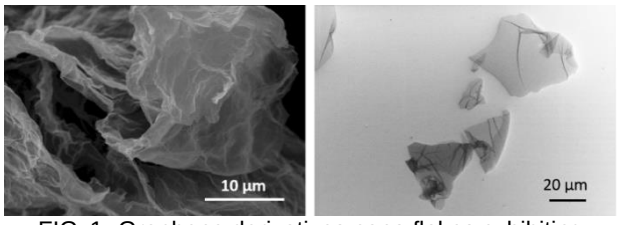

FIG. 1. Graphene derivatives nano flakes exhibiting different morphology, shape and properties as received.

For immobilization process zirconia surfaces were at first activated by using aminopropyl diisopropyl ethoxysilane (APDS). For the APDS-activated surfaces exhibiting –NH_2 active groups, additional catalysts, EDC and NHS were applied to reinforce the reaction between -NH_2-functionalities and activated –COOH-groups of graphene-derivatives. Two different techniques, drop casting and immobilization from graphene solution by immersing of the samples in the shaking system to avoid sedimentation, were performed. At the end, the samples were evaluated regarding to their morphology, graphene-derivatives coupling behaviour and the stability of the obtained graphene-layers by using SEM analysis. Additionally AFM measurements evaluated the morphological profile of the coated samples and determined the thickness of GO-coatings.

Results and Discussion
The cell culture tests approved the cytocompatible behaviour of all selected graphene nano-flakes before immobilization, since more than 95% of cells were viable after incubation time. It was shown, that owing to the catalysts a higher amount of graphene-derivatives could be found on the ceramic surface. The stability of the coatings was established via ultra-sonication treatments. All graphene-derivates could be immobilize to the activated ceramic surface. The most promising results were obtained by using graphene oxide obtained from exfoliation of Asbury 1 (GO A1). By using drop casting method a multilayer of GO A1 was attached to the surface, while during spontaneous immobilization from the graphene-solution a well distributed, surface-coverage self-assembled graphene oxide monolayer could be obtained (FIG. 2). AFM evaluation have confirmed that the ceramic surface was well covered with a thin layer of well distributed graphene nano-flakes. The thickness of obtained coating varied from 3-12 nm.

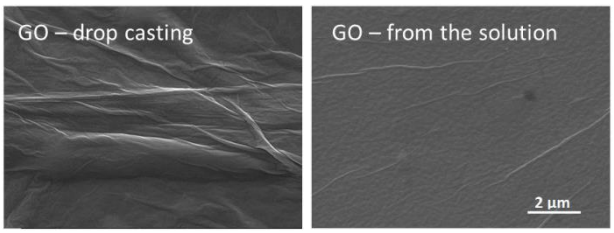

FIG. 2. Coating of graphene oxide on the silanized zirconia substrates by using drop casting method (left) and spontaneous immobilization from the graphene solution, which resulted in well distributed, surface-coverage graphene oxide monolayer (right).

Conclusions
In our study, the immobilization of graphene derivatives by using different methods was successfully performed. We could obtain well coved thin GO layer on ceramic surface.
Through this technique, the properties of graphene-derivatives and zirconia could be merged to create a versatile biomaterial. Moreover, through highly reactive graphene oxide additionally biological agents such as proteins, enzyms, antibacterial agent or drugs could be immobilized on the graphene-derivatives-modified substrates to reinforce its biological activity or enhance other functionalities according to application requirements.

Acknowledgments
Funded by the Excellence Initiative of the German federal and state governments (Grant Nr. OPSF456).

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BIOLOGICAL PROPERTIES OF FIBROUS MEMBRANES MODIFIED WITH NATURAL AND SYNTHETIC ANTIBIOTICS

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ENGINEERING OF BIOMATERIALS 153 (2019) 40]

Introduction

Regenerative medicine and tissue engineering for scaffolds preparation focus on achieving the most accurate reproduction of the native environment, which is beneficial for cell proliferation. However, because of the specificity of the process of seeding cells on the scaffold as well as the subsequent implementation of biomaterial in damaged tissue the concurrent processes must be taken into consideration that always accompanies the adhesion of cells - bacterial colonization and the formation of bacterial biofilm on the surface of the scaffold. Thus, a permanent or temporary scaffold should have microstructure and physicochemical properties similar to the extracellular matrix (ECM), stimulating cells to initiate repair processes with a high degree of air permeability and suitable properties reducing the possibility of bacterial infection [1].

The emergence of antimicrobial resistance (AMR), has focused attention on the searching of natural products continue to provide new chemical structures with high levels of antibacterial activity.

There are many literature reports informing about the antibacterial, anti-inflammatory and antioxidant activity of curcumin [2,3].

In this work examples of natural (curcumin) and chemical (gentamycin) bioactive molecules that have been loaded on polymeric electrospun fibers are presented. This molecules highlighting the antibacterial properties and their capable of enhancing the healing process (anti-inflammatory molecules).

Materials and Methods

The PLA 3251D polylactide from Nature Works was used in the research as a base material for electrospinning. Analytically pure reagents provided by Avantor SA: dichloromethane (DCM), dimethylformamide (DMF), were used as solvents for the preparation of spinning solutions. polymer fibers were modified with gentamicin (Polfa SA), in the form of gentamicin sulfate and Curcumin (from Curcuma longa (Turmeric), Sigma Aldrich). The measurement of diameters of PLA submicrofibers with additives was made using the scanning electron microscope NOVA NANO SEM 200. Surface wettability of the tested materials was determined by means of direct measurements (DSA 25E, Kruss) at room temperature using high purity water (UHQ, PURE Lab, Vivendi water) as a measuring liquid. The free surface energy was determined by the Owens-Wendt method using diiodomethane as a non-polar liquid. The durability of biomaterials (scaffold) was tested in in vitro condition: PBS/37°C/CO_2. The presence of biomolecules in membranes were confirmed by FTIR. Antibacterial properties were checked in contact membrane with E.coli (Hilton Muller test). The basic biocompatibility of the fibrous scaffolds (cytotoxicity, viability, adhesion to the scaffold) in contact with the fibroblasts and macrophages were tested.

Results and Discussion

Non-woven fibrous mats can be successfully modified with natural or synthetic active biomolecules. Additives have impact on fibers morphology. Expected result on cellular response: biocompatible materials for curcumin and gentamicin. Antimicrobial activity depends on concentration of bioactive molecules in polymer solution.

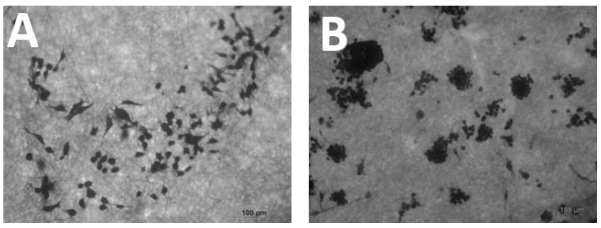

FIG. 1. Macrophages on PLA membranes with: A – gentamycin, B – curcumin, after 3 days magnification 20x.

Conclusions

The microstructure of fibrous membranes can provide unique microenvironment to cell proliferation. The antibiotic content in the fiber inhibits bacterial growth. Both materials pose a potential wound patch dressing for dermis and epidermis defects.

Acknowledgments

This work was performed within the framework of funding for statutory activities of AGH University of Science and Technology in Krakow, Faculty of Materials Science and Ceramics (11.11.160.182).

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SURFACE MODIFICATIONS USED FOR INFLOW CANNULAS OF THE VENTRICULAR ASSIST DEVICES – STATE OF THE ART

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[ENGINEERING OF BIOMATERIALS 153 (2019) 41]

Introduction
Nowadays, the Mechanical Circulatory Support (MCS) within the Ventricular Assist Devices (VAD) [1] appears to be a reliable and effective solution for patients with advanced heart failure (HF). After many years of work extracorporeal pulsatile VAD's are replaced by new generations of fully implanted continuous flow (CF) pumps. Clinical experience has shown that actual pump constructions still need to be improved to minimize the risk of complications during heart assistance.

Materials and Methods
One of the complications is the inflow obstruction, caused by the ingrowth of tissue into the light flow, and pump thrombosis [2,3]. The main goal is to develop coating for external surface of the inflow cannula to provide controlled tissue ingrowth. The smooth surface of the cannula results in tissue overgrowth into the light flow and may be a source of emboli. The paper presents the inflow cannula's surface modifications performed by different VAD manufacturers within the topography characterization.

Results and Discussion
The inflow cannulas used in CF VADs are mainly made of titanium alloy due to its mechanical properties and high biocompatibility. In general discussed surface coatings were characterized by roughness of about ≈ Ra=15μm, high porosity ≈82% and well wettability ≈60°. The surface was covered with titanium microspheres or titanium mesh.

Conclusions
The developed surfaces and clinical experience confirm the possibility to control the tissue ingrowth of the external surfaces of the inflow cannula on the tissue-implant interface.

Acknowledgments
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RH-ROT/266798/STRATEGMED-II

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PLA/HAP MICROFIBERS INCORPORATED GRAPHENE-LOADED HYDROGELS FOR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 42]

Introduction

In tissue engineering, it is especially important to design scaffolds which mimic the complex, multi-scale structure of natural tissues. In recent years, polymer hydrogels (polymer matrices able to absorb a large amount of water) have gained a lot of research interest [1-3] due to their similarity to the extracellular matrix (ECM).

The applicability of chitosan-based hydrogels, despite their superior biological properties, is limited by poor mechanical properties and stability. A multi-scale scaffold based on chitosan (CS) hydrogel with incorporated microfibers was proposed in this study as a solution to this issue. The advanced electrospinning (ES) method was chosen for the fabrication of poly(lactic acid)/hydroxyapatite (PLA/HAp) nonwoven. In addition, to enhance the hydrogel properties, graphene-based materials (GO or rGO) and tannic acid (cross-linker, TAc) were introduced to the polymer matrix.

Materials and Methods

CS (High Mw, DD >90%) and sodium tripolyphosphate (TPP) were obtained from Acros Organics, USA. Lactic acid (LAc, 88%), TAc, NaOH, NaCl, N,N-dimethylformamide (DMF) and dichloromethane (DCM) were purchased from Avantor Performance Materials Poland S.A. PLA was obtained from NatureWorks LLC, USA. HAp was obtained from Chema-Elektromet, Poland. Graphene oxide (GO) and reduced graphene oxide (rGO) were prepared by ITME, Poland.

The PLA microfibers modified with bioactive particles (HAp) were fabricated by an electrospinning method (ES). Polymer solution (13% w/v) was obtained by dissolving PLA in binary solvent system of DCM and DMF (2.5:1 v/v). The concentration of inorganic particles was 6 wt%. The parameters of ES were optimized (temperature: 50°C, the gap between the tip of the needle and the collector: 4 cm, humidity: 10%, voltage: 25 kV) to obtain fibers with specified microstructure. In the next step, fibers were introduced to the hydrogel matrix (5% wt. CS in 5% LAc, with 10% wt. TAc and 0.5% wt. GO or rGO) to create three-dimensional, multi-scale scaffolds. Samples were frozen in molds for 24 h. Next, they were immersed in a gelling solution (5% NaCl and 0.5% TPP, 24 h) at 4°C [4].

FTIR-ATR, XRD, XPS and SEM methods were used to characterize graphene materials and fabricated scaffolds. Also, thermal (DSC), mechanical (compression test) and rheological properties were examined. The biocompatibility of hydrogels and fibers was evaluated by culturing MG-63 cells in direct contact with the materials.

Results and Discussion

The microstructure of the PLA/HAp microfibers and CS-based tube scaffold was observed using SEM (FIG. 1).

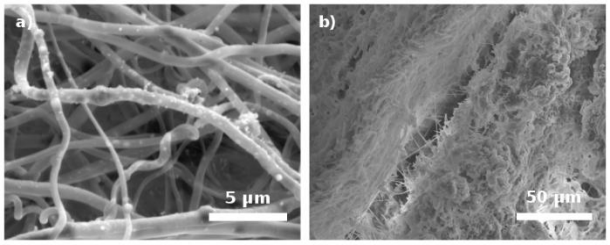

FIG. 1. SEM images of PLA/HAp microfibers (a) and CS/GO scaffold with incorporated woven (b).

The ES process allowed to successfully fabricate randomly oriented microfibers. The main aim of incorporation nonwovens into CS-based matrix was to increase the mechanical properties and degradation time of CS/GO and CS/rGO hydrogels. Also, the three-dimensional tubes exhibited unique morphology with two types of pores. Gaps between scaffolds walls can potentially improve cells penetration and transport of nutrients and metabolic wastes.

In addition, composites with microfibers exhibited improved stability during PBS immersion test (37°C, 6 weeks) and high bioactivity (SBF, 37°C, 2 and 4 weeks). *In vitro* test (MG-63) showed good cytocompatibility of all the samples.

Conclusions

A novel method was developed to fabricate three-dimensional scaffolds in the form of tubes dedicated for cartilage and bone tissue engineering. Dual porous microstructure of the samples can potentially improve cells penetration into the scaffold. Multi-scale scaffolds with hydrogel matrix were created to better mimic the complex microstructure of the ECM.

Acknowledgments

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BIOPOLYMERIC SCAFFOLD FOR CELL VISUALISATION IN 3D ENVIRONMENT USING COHERENCE-CONTROLLED HOLOGRAPHIC MICROSCOPY

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[ENGINEERING OF BIOMATERIALS 153 (2019) 43]

Introduction
Coherence-controlled holographic microscopy (CCHM) is an emerging single-shot imaging technique used for fast processes visualisation [1]. Unlike fluorescence microscopy, which is currently one of the most used bioimaging technique, CCHM is a non-invasive, label-free technique with the ability to visualise cells in real-time. A challenging aspect of this technique is light scattering as the imaging in CCHM is based on the interference of the object and the reference light beams, which enables to detect the phase delay of light transmitted through the specimen.= [2]. Cells are overall weakly scattering absorbing specimens and highly scattering environment, such as polymeric substrates, can distort final images. Therefore till this day, CCHM has been successfully used mostly in visualising cells in a 2D environment even tough 3D visualisation represents physiological environment better [3,4].
Within the context, the presented work aimed to establish a microstructured scaffolding material for visualisation of cellular interaction in a 3D environment using CCHM.

Materials and Methods
The 3D microstructure of biopolymeric scaffolds was achieved using the process of freeze-drying. The stability upon disintegration in the environment of the culture medium was improved using chemical crosslinking reaction initiated by carbodiimides. Microstructure in dry state was visualised and evaluated using scanning electron microscopy (SEM). Optical transparency was evaluated using UV-VIS spectroscopy at 600 nm. Visualisation using CCHM was achieved at 37°C and 5% CO_2 atmosphere. The cell line of normal human dermal fibroblasts nHDF was maintained in Eagle's minimal essential medium supplemented with 10% fetal bovine serum.

Results and Discussion
The 3D microstructured biopolymeric scaffolds, fabricated in our study, have the advantage of high optical transparency resulting in minimal light scattering effect. Biopolymeric nature of the scaffolds simulates extracellular matrix by its chemical composition, therefore creates an environment that closely represents the physiological environment. Optimal concentration of biopolymeric substances was set to 0,2 % (w/w). At this concentration, the scaffolds were still easy to be manipulated with as well as had a minimal negative effect to light scattering. SEM visualisation revealed' microstructure of the scaffolds having high porosity and inhomogeneous pores. The cellular behaviour was investigated using CCHM in order to determine the effect of prepared biopolymeric scaffolds on cultured cells adhesion, alignment, orientation and morphology in comparison to glass microscope slides. The cultured cells were investigated at different time intervals to monitor their behaviour. Cells were able to attach and align to biopolymeric fibres within the microstructure of the scaffolds without forming a cluster or unaccustomed morphology. After addition of model presumably toxic substance, there were visible changes in cellular behaviour and morphology.

Conclusions
In presented work, we have achieved preparation and characterisation of microstructured biopolymeric scaffold with low optical density designed for the visualisation of cellular interactions in the 3D environment using CCHM.

Acknowledgments
This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and by Special Research project of Brno University of Technology under no. CEITEC VUT-J-19-6086.

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3D BIOPRINTING HANDHELD TOOL CONCEPT FOR INNOVATIVE OSTEOARTHRITIS TREATMENT WITH STEM CELLS UTILIZATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 44]

Introduction

Osteoarthritis (OA) is the most common chronic joint disease involving progressive damage and loss of cartilage, remodelling of subchondral bone, osteophyte formation, weakening of periarticular muscles and thickening of the joint capsule[1]. Over the next decade, the number of people affected by OA is expected to double due to population ageing and increased rate of obesity. Novel nanocomposite responsive materials combined with adipose tissue-derived stem cells (ASCs) and a remotely controllable ultrasound (US) treatment is developed within the H2020 project. Materials and cells will be delivered in situ through an innovative handheld 3D bioprinter, introduced to the operating area during an arthroscopic treatment through available port.

Materials and Methods

Before the pneumatic flow for bioprinting preliminary concept design, two different materials were tested in order to analyse the extrusion parameters to sketch structural features of 3D bioprinting device: PEG-fibrinogen - Pluronic and PBS - Pluronic based thermosensitive and UVA curable hydrogel precursors (manufactured by: REGENTIS Biomaterials, Israel).
The material in the volume of 5 ml was storage up to room temperature (20-22°C) and stabilized within 1 hour. Different needle's diameter dimensions were used 0,4; 0,6; 0,8 and 1,0mm. The following parameters were evaluated: pressure force on the syringe plunger necessary to begin the extrusion process as well as the extrusion velocity (ml/min) in the constant force with reference to the needle diameter.
Basing on the test results the first 3D bioprinting handheld device preliminary concept was designed to print the nanocomposite hydrogel and the ASCs directly onto the cartilage site to be treated – lesions in advance processed by the surgeon during an arthroscopic procedure.

FIG. 1. 3D Bioprinting Handheld Tool Concept For Innovative Osteoarthritis Treatment.

Results and Discussion

Characteristic showing dependence of the velocity of hydrogel extrusion versus pressure force have allowed to evaluate the extrusion time in regard to tip diameter and choose the proper parameters of the cartridge chamber mechanism for 3D bioprinting tool. The 3D handheld bioprinter preliminary concept was developed including: chambers (cartridges), control unit, driving method, handheld device and single-purpose extrusion tips.
The number of chambers/cartridges (nanocomposite hydrogel without cells, nanocomposite hydrogel with cells embedded, the primer) were defined together with the main features: cylindrical shape, volume up to 10ml. The chambers are made from biocompatible material and easily pluggable within the handheld device.
An additional tool holding a camera and a light source will provide UV light in situ to promote hydrogel crosslinking.
Cells could be mixed with the nanocomposite hydrogel before loading the cartridge, or within the instrument, before printing. A deep investigation of the hydrogel/cell mixing mechanism within the arthroscopic tool will be carried out in the future to choose one of the possible strategies: targeting the formation of a core-shell structure, in which cells are in the core and the crosslinked hydrogel constitutes the external shell of the structure or targeting a different and novel printing strategy and structure architecture.
Control unit is expected to be equipped with ergonomic and usable user interface. Working parameters values (START/STOP, flow rate [%], active tool and auxiliary device activation e.g. light source) are going to be presented on the interface in a transparent and clear way to be easily recognized by the operator.
The handheld device is controlled by firmware installed in microcontroller to execute a particular printing process and also enabling presentation of printing-related functions such as initialization, direct motion control, printing start/stop.
Consequently, the device dimensions are assumed to be designed to be as versatile as possible respect to the different hydrogel viscosity values that may be obtained, keeping in mind the shear stress level harmless for stem cells survivability.
Extrusion tips were designed to deposit the bio ink with right shape and diameter without exerting excessive stress to cells (max. 15-25 kPa). For such reason, clogging inside the nozzle tip must be avoided and the flow was optimized considering the diameter of the tip. Fabrication of tolerances on the nozzle is important, and for each different dispensing tip mounted, calibration of the valve may be needed, especially for very long-dispensing tips.

Conclusions

In the next project stage, the hydrogel which confirms good results will be extruded with ASCs, repeating the experiments for comparing the pressure force and the extrusion velocity.
The prototype of bioprinting handheld system will be developed as a device which should assist the surgeon in depositing the bio inks during arthroscopy in a well-controlled shape according to the patient's cartilage anatomy. The prototype will be evaluated within the in-vitro and in-vivo test.
Then the final bioprinting handheld system will be designed and tested in the usability tests on human volunteers.

Acknowledgments

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GRAPHENE OXIDE-BASED BIOMATERIALS AS A POTENTIAL TOOL FOR CARTILAGE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 45]

Introduction

Currently, combining biomaterial scaffolds with living stem cells for tissue regeneration is the main approach for tissue engineering. Human adipose mesenchymal/stromal stem cells (hAT-MSCs) are promising candidates for tissue regeneration through pro-angiogenic, immunomodulatory and anti-apoptotic activity [1] as well as differentiating into specific tissues, in particular bone and cartilage [2].

Graphene Oxide (GO) is a biophysical phenomenon that accelerates biological processes by several orders of magnitude [1,2]. Herein, we ventured to assess the influence of several compositions of GO-based hybrid biomaterials on chondrogenic and osteogenic differentiation [3] by comparing *in vitro* cell cultures conditions and selected markers expression of hAT-MSCs grown under 2-dimentional or 3-dimentional cell culture conditions.

Materials and Methods

hAT-MSCs, obtained from adult young male and female donors, were seeded at density of 4,200 cells/cm^2 for 2D culture and 30,000 cells/micromass for 3D culture. Both cultures, 2D and 3D, were expanded in a standard growth medium (αMEM, Macopharma; 10% human plated lysate, Macopharma) on cell culture plates, 6-well (Eppendorf) coated with 10 µg/cm2 dedicated GO-buffer. Subsequently, cells were collected in 3, 7 and 14 days. Medias were changed every 3-4 days. Changes in cell morphology between defined time point frames were carried out using phase-contrast microscopy (Nikon Eclipse TS100) with MicroPublisher Camera (Qimaging). To assess the induction of chondrocyte differentiation, the cells were fixed using 4% paraformaldehyde (POCH) and stained with 1% Alcian Blue 8G (Merck Millipore) up to 30 min. The cells were observed under phase-contrast microscope (Nikon Eclipse TS100).

RNA isolation was performed using the Universal RNA / miRNA Purification Kit (EURx). Reverse transcription was carried out using the NG dART RT kit (EURx). Analysis of the expression of selected genes (normalizing and characteristic for cartilage and bone cells) was performed by means of real-time PCR analysis.

Results and Discussion

Our preliminary studies show differences between hAT-MSC 2D and 3D culture in the tested media including differences in morphology and deposition of cartilage matrix proteoglycans depending on tested GO-based biomaterials. Quantitative analysis of gene expression revealed these observations. Part of tested GO-based scaffolds may enhance hAT-MSCs differentiation toward chondrogenic and osteogenic cells *in vitro*. Thus, these data may suggest, combining hAT-MSCs with appropriately biofunctionalized biomaterial such as GO-based scaffolds, on the one hand, provides them with a niche for growth, and on the other, it can increase their biological potential, due to which we will obtain more effective implants for the treatment of bone-cartilage defects.

Conclusions

Obtained preliminary results may indicate positive effect of GO-based biomaterials on chondrogenic and osteogenic differentiation. Our data allow us to choose most promising GO-based biomaterials for the purpose of preclinical application.

Acknowledgments

The study was funded by National Centre for Research and Development grant STRATEGMED 3 (STRATEGMED3/303570/7/NCBR/2017).

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STUDIES OF PHOTOCATALYTIC PROPERTIES AND BIOACTIVITY OF TITANIUM-OXO CLUSTERS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 46]

Introduction

The unique physicochemical and biological properties of titanium dioxide favour its wide application in various fields of our life. Especially, the photocatalytic properties and bioactivity of materials based on TiO_2 are intensively studied in recent time [1,2]. Titania photoactivity is utilized in water splitting, air and water purification, environmental pollutants reduction, and in antimicrobial applications. However recently, considerable attention is devoting to the use of titanium(IV) oxo-complexes (TOCs) as systems exhibiting similar properties to TiO_2. The results of previous studies revealed significant importance of TOCs in the synthesis of inorganic-organic composite materials, which are produced through introduction of metal oxo-clusters into the polymer matrix [3]. The possible interactions between inorganic and organic components may result in an improvement of structural properties of the polymer, as well as of its thermal and mechanical ones due to crosslinking and filling. The unique properties of oxo-clusters, e.g. photochromicity, photocatalytic/biological activity can give completely new properties to the composite material in comparison to the base polymer [3]. Therefore, the studies on TOCs synthesis of the titanium-oxide core of the desirable architecture, size, physicochemical properties and their bioactivity, are important [4-6]. Especially, the studies on the structural conversion of Ti(IV) multinuclear oxo-complexes containing $\{Ti_a\text{-}(\mu_i\text{-}O)_b\}$ cores are relevant for their controlled synthesis [7-9]. The purpose of our research works is to optimize synthesis conditions of Ti(IV) oxo-complexes containing functionalized carboxylate groups and their structure determination. The estimate the photocatalytic properties and bioactivity of composites produced by dispersion of TOCs in polymeric matrixes is an important part of our works.

Materials and Methods

Multinuclear oxo-complexes were synthesized in the direct reaction of the titanium(IV) alkoxides and organic acids in different molar ratios and solvents using standard Schlenk techniques under Ar atmosphere and in room temperature. The slow evaporation of the reaction liquors under the inert gas (3-5 days), led to the isolation of crystalline products. Single crystal X-ray diffraction studies allowed to solve the structure of crystals, which quality was suitable. The structure of the remaining produced oxo-complexes was determined basing the analysis of spectroscopic data (IR, Raman, MS, NMR). Photocatalytic activity studies were carried out basis on the studies of the UV-Vis induced degradation processes of organic dyes, stearic acid and acetone. In order to evaluate of antimicrobial activity, LIVE/DEAD, Alamar Blue staining and CFU method were used.

Results and Discussion

The direct reaction of the titanium(IV) alkoxides ($Ti(OR)_4$, R = ^{i}Pr, ^{i}Bu) and functionalized organic acids (4:1 alkoxide/acid molar ratio, standard Schlenk techniques, inert atmosphere, room temperature) led to the formation of $[Ti_3O(O^iPr)_8(O_2CR')_2]$ and $[Ti_4O_2(O^iBu)_{10}(O_2CR')_2]$ (R' = $PhNH_2$, PhCl, $PhNO_2$, $C_{13}H_9$, C_4H_7) complexes [9,10]. The molecular structure of $\{Ti_aO_b\}$ cores, which were found in the structure above mentioned compounds, determined using single crystal X-ray diffractions, are presented in FIG. 1.

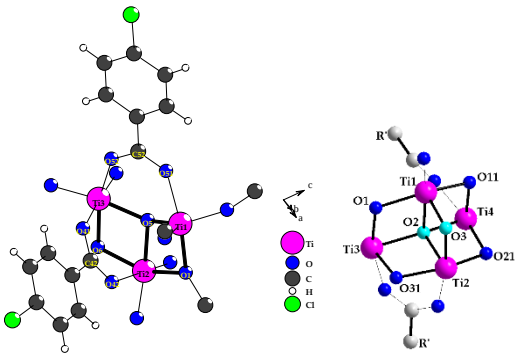

FIG. 1. The structures of $\{Ti_aO_b\}$ (a = 3, 4, b = 1, 2) cores, which was found in $[Ti_3O(O^iPr)_8(O_2CR')_2]$ and $[Ti_4O_2(O^iBu)_{10}(O_2CR')_2]$ (R' = $PhNH_2$, PhCl, $PhNO_2$, $C_{13}H_9$, C_4H_7) complexes (crystallographic ball-stick scheme). For clarity, the terminal alkoxide groups are omitted.

The type of the $\{Ti_aO_b\}$ core and the carboxylate group allows to modulate the band-gap of synthesized compounds in the range of 3.6 – 2.0 eV. The Ti(IV) oxo-complexes were applied in fabrication of poly(methyl methacrylate)/TOCs composite materials (the oxo-complex content - 20%). The highest photocatalytic activity was evidenced for these composites, which contain fluorene ($-OOCC_{13}H_9$) and $-OOCPhNH_2$ groups in the TOCs structure. Simultaneously, the results of microbiological tests revealed that the best biocidal activity (*S. aureus*, *E. coli*, and mixtures of mold spores) was noticed for polymer/TOCs composite coatings, which contain the $-OOCC_{13}H_9$ and $-OOCPhNH_2$ ligands.

Conclusions

The results our works allowed to optimize the synthesis conditions of three- and tetranuclear Ti(IV) oxo-complexes ($[Ti_aO_b(OR)_c(OOCR')_2]$ (a = 3, 4, b = 1,2, c = 8,10; R = ^{i}Pr, ^{i}Bu, R' = $PhNH_2$, PhCl, $PhNO_2$, $C_{13}H_9$, C_4H_7)). The dispersion of the produced TOCs in the polymer solutions made it possible to the formation of polymer/TOCs composite coatings. The use of TOCs, which contain fluorene and -OOCPhNH2 ligands, in synthesis of polymer/TOCs systems allows to receive the coating materials with suitable photocatalytic properties and microbicidal activity.

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NOVEL MULTICOMPONENT ORGANIC-INORGANIC WHEY PROTEIN ISOLATE (WPI)/GELATIN/CALCIUM PHOSPHATE-HYDROGEL COMPOSITES FOR BONE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 47]

Introduction

The present work focuses on the development of novel multicomponent organic-inorganic hydrogel composites for bone tissue engineering. A combination of the organic components commonly used in food industry, namely whey protein isolate (WPI) and gelatin from bovine skin, as well as inorganic material commonly used as a major component of hydraulic bone cements, namely α-TCP in various concentrations (0-70 wt.%) was proposed.

Materials and Methods

WPI (BiPro, Davisco Foods International Inc., USA), 97.7% protein, 75% beta-lactoglobulin by dry mass, and gelatin from bovine skin type B (Sigma-Aldrich, UK) were used. α-TCP was produced by a wet chemical method. To produce composites, 40 wt./vol.% WPI solution was mixed with gelatin powder (20 wt%) in ultrasonic bath (40°C) for 30 min. Warm WPI/gelatin solution was mixed with α-TCP powder in 2 mL Eppendorf tubes for 30 s. Closed tubes were immersed in cold (-20°C) ethanol to induce fast gelation. After 2 minutes, tubes were immediately heated at 100 °C for 5 min to induce WPI thermal crosslinking, then autoclaved (121°C for 30 min). α-TCP concentrations of 20, 30, 40, 50, 60, and 70 wt% were compared. Human osteoblast-like MG-63 cells (Sigma Aldrich, USA) were seeded on materials in a concentration of 10.5×10^3 cm^{-2} in 1 ml of culture media. Metabolic activity was assessed by the MTS assay.

Results and Discussion

α-TCP underwent incomplete transformation to calcium-deficient hydroxyapatite (CDHA). The values of Young's modulus and the stresses corresponding to compression of a sample by 50% increased almost linearly with increasing concentration of ceramic phase (FIG. 1). Microcomputer tomography showed inhomogeneous distribution of the calcium phosphate (CaP) phase in the resulting composites (FIG. 2). In vitro studies revealed that the composites support adhesion, spreading, and proliferation of MG-63 cells (FIG. 3).

Conclusions

The composites show potential as materials for tissue regeneration and can potentially be processed into 3D scaffolds using a low temperature 3D-printing technique.

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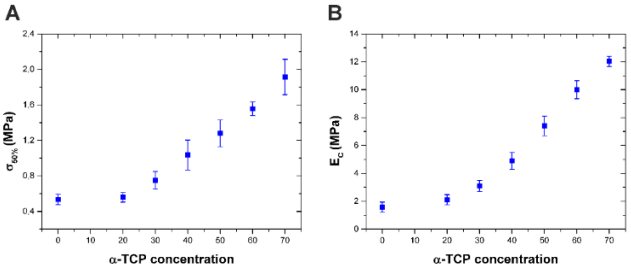

FIG. 1. Compressive strength at 50% strain σ50% (A) and compressive modulus EC (B) of the WPI/gelatin/CaP hydrogel composites.

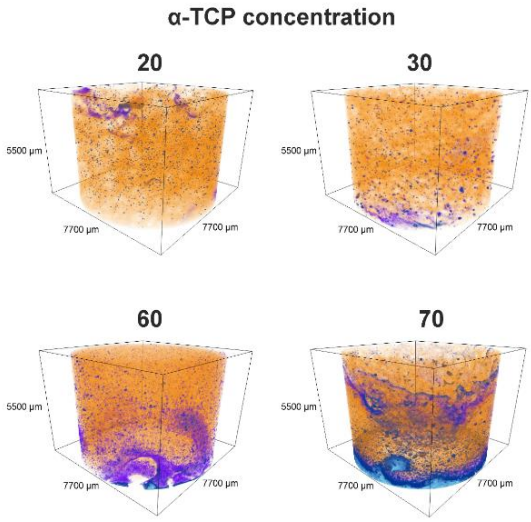

FIG. 2. µCT analysis of the WPI/gelatin/CaP hydrogels - 3D rendering.

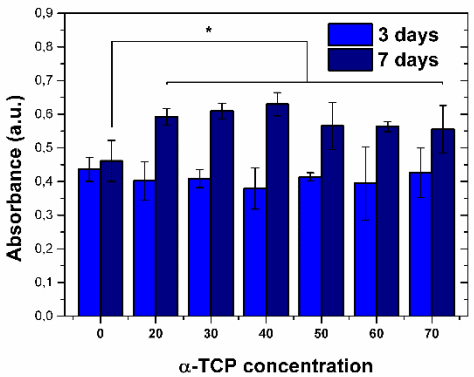

FIG. 3. Metabolic activity of MG-63 cells cultured for 3 and 7 d in direct contact with composites. Statistically significant differences ($p < 0.05$) relative to the hydrogel unmodified with CaP are indicated by asterisk * (differences detected only at 7 d).

WHEY PROTEIN ISOLATE COATINGS FOR BIOMATERIALS

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[Engineering of Biomaterials 153 (2019) 48]

Introduction

Whey protein Isolate (WPI) is a by-product of the dairy industry. Its main component β-lactoglobulin is able to assemble into fibrils with functional properties that can serve as a new coating material for biomaterial surfaces. The aim was to develop sterile fibrillar coatings on glass substrates and assess the effect of the coatings on growth, morphology and differentiation of human bone marrow stromal cells (hBMSC). Three sample groups were compared: (i) uncoated glass and glass coated with fibrils formed at (ii) pH 2 and (iii) pH 3.5.

Materials and Methods

For the fibril formation a 2.5 wt% WPI (BiPro, Davisco Foods International Inc., USA) solution was incubated at 90ºC for 5 h under stirring conditions at a low pH (2, 3.5). The fibrillar suspension was placed on glass coverslips of diameter 1 cm to allow adsorption of fibrils to the surface. After rinsing and drying, glass substrates coated with fibrils were autoclaved at 121ºC for 15 minutes. Coating morphology was assessed using scanning electron microscopy (SEM). hBMSC (primary osteoblast-like cells) were seeded at a density of 5,555 hBMSC/cm² in DMEM with 10% heat-inactivated fetal calf serum with antibiotics (BM), from day 4 in BM with 10 mM β-glycerophsosphate and 300 µM ascorbate (OM/D); medium was changed twice per week. Metabolic activity was assessed using the MTS assay on day 2 & 4, cell morphology by immunofluorescence staining 2 h after seeding, differentiation by activity of tissue non-specific alkaline phosphatase (TNAP) on day 11.

Results and Discussion

SEM confirmed the presence of fibrillary coatings after autoclaving (FIG. 1). Cell adhesion was observed on all sample groups, but spreading and staining intensity were superior on coated samples (FIG. 2). TNAP activity was observed on all sample groups (FIG. 3).

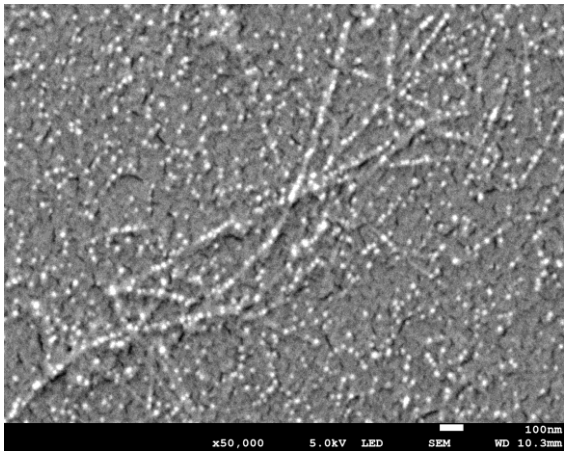

FIG. 1. SEM image of WPI fibrillary coating formed at pH 2 after autoclaving. Scale bar = 100 nm.

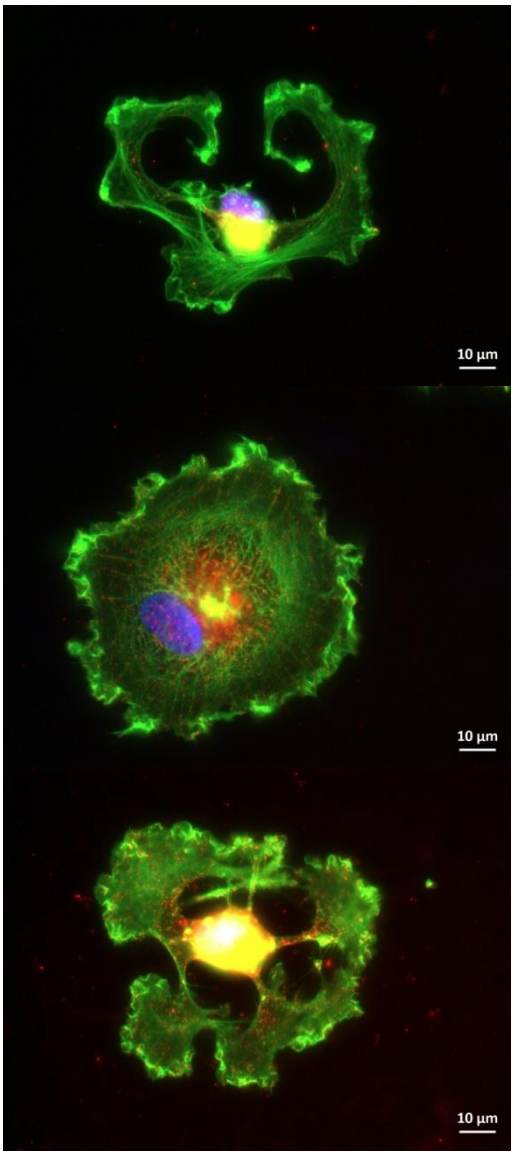

FIG. 2. Morphology of hBMSC on glass (top), fibrils formed at pH 2 (middle) and pH 3.5 (bottom), 2 h after seeding. Scale bar = 10 µm.

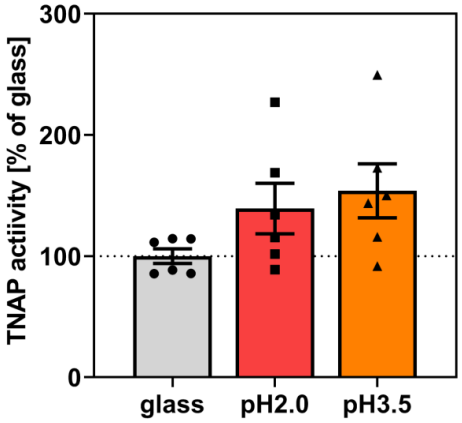

FIG. 3. TNAP activity in hBMSC cultured for 11 days in differentiation medium, n=3.

Conclusions

Coatings withstand autoclaving and support adhesion and differentiation of hBMSC. This paves the way for enhancement of the fibrils with further biomolecules.

Acknowledgment

N8 Agrifood grant "Food2Bone" (T.E.L.D.), German Research Foundation (DFG), SPP 1934 DiSPBiotech (project number 273937032) (J.K.K.).

TITANIA NANOTUBES AS SCAFFOLDS WITH OPTIMAL NANOTOPOGRAPHY FOR ADIPOSE-DERIVED STEM CELLS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 49]

Introduction

Since bone tissue is composed of various components in nanoscale, a surface, which mimics such structural hierarchy and provides cues at the nanoscale may further improve the response of cells on the surface to enhance bone formation and improve long term stability of the implant. Thus far, nanosurfaces have not been implemented for joint implants, however, many studies are currently directed towards understanding how nanoscale topographies on Ti/Ti alloy surfaces can improve implant integration. Some nano-topographies that have been explored in the research are nanofibers [1], nanopores [2] and nanotubes (TNT) [3]. Studies have shown that Ti/Ti alloy surface with a nanotopography promotes fibroblast and osteoblast cells adhesion and proliferation and creates an ideal environment for osteogenesis.

TNT (titania nanotubes) fabricated on the surface of titanium alloy using an anodization technique, provide a template with a hierarchy similar to that of natural tissue and have been shown to alter cellular functionality on the surface similar to that of natural tissue. However, the optimal size of nanosurfaces to promote cell adhesion, proliferation, and differentiation is still disputed. Because stem cells are important in the healing process, it is essential to study the response of stem cells on these nanostructured surfaces in vitro.

In all tissues of the body, stem cells become activated when an injury occurs and are recruited to the injury site to aid in the tissue repair process. When a biomaterial is implanted, the body reacts similar to an injury and stem cells are recruited to the implant site. Since, stem cells play an important role in tissue repair in the body, their interaction with biomaterials is critical for the long-term success of medical devices. Adipose-derived stem cells (AD-MSC), which are mesenchymal stem cells obtained from adipose tissue, have been identified as a putative population of multipotent stem cells, easily accessible, and available in large numbers. This fact makes them an attractive source for studies on evaluation of stem cell interaction with biomaterials. To date, very few studies have investigated the adhesion, proliferation, and differentiation of AD-MSC on TNT surfaces. Such research is the goal of our scientific activities.

Materials and Methods

In order to fabricate titania nanotube layers (TNT) on the surface of Ti6Al4V substrates, the electrochemical anodic oxidation method was used. The first generation nanotubes were fabricated using an aqueous electrolyte solution - 0.3% HF and different anodizing potential values (5 - 40 V). Afterwards, studies were conducted to determine the adhesion and proliferation rate of adipose-derived mesenchymal stem cells, cultured *in vitro* on oxide scaffolds on the surfaces of titanium implants. The degree of adhesion and proliferation of AD-MSC cells was evaluated using MTT (mitochondrial enzyme activity) after 24 and 72 hours. The above studies were carried out using a commercially available line of unmodified human AD-MSC cells. Biocompatibility of biomaterials was also assessed on the basis of the degree of integration of MG-63 osteoblasts and L929 fibroblasts cultures on their surface *in vitro*. Additionally, on the nanocoatings' surfaces a co-culture of (a) AD-MSC cells with L929 fibroblast cells, (b) AD-MSC cells with MG-63 osteoblast cells were formed. The degree of adhesion and proliferation of cell co-cultures was evaluated using MTT in various time variants (after 24 and 72 hours). The above studies were supplemented by the morphology analysis of AD-MSC, L929 fibroblasts and MG-63 osteoblasts cells, using scanning electron microscope (SEM).

Results and Discussion

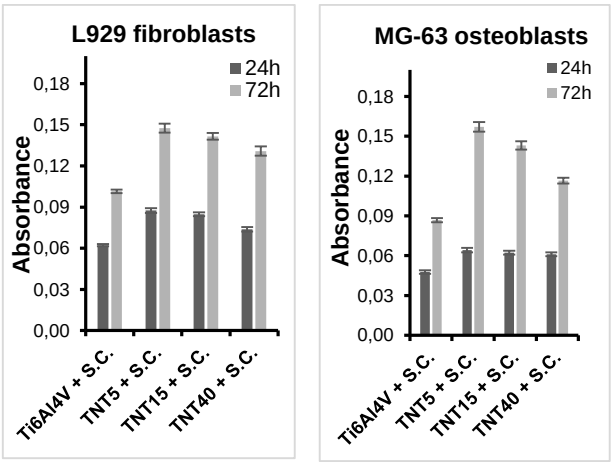

FIG. 1. Murine L929 fibroblasts and human MG-63 osteoblasts proliferation (after 24h, 72h) on the surface of Ti6Al4V and Ti6Al4V/TNT5-TNT40 samples enriched with AD-MSC, detected by MTT assay. The absorbance values are expressed as means ± SEM of four independent experiments.

Based on the results obtained from the MTT test, with an increase of incubation time more AD-MSC cells, L929 fibroblasts, as well as MG-63 osteoblasts proliferated on the surfaces of all tested biomaterials. Ti6Al4V/TNT nanocoatings provoked a significant increase in cells proliferation compared to the reference Ti6Al4V alloy. As it can be seen in FIG. 1, the nanoporous scaffoldings are also conducive to the interaction between different cell types.

Conclusions

On the basis of the obtained results, we observe that the nanoporous surface of implants is a conducive substrate for the integration and proliferation of AD-MSC cells, fibroblasts, osteoblasts, as well as cellular co-cultures, and thus promotes future bone formation.

Acknowledgments

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CARRAGEENAN OLIGOSACCHARIDES UNDER EXTERNAL FORCES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 50]

Introduction

Carrageenans are a group of water-soluble sulfated-D-galactans extracted from marine red algae (*Rhodophyceae*).[1] The main field of application of carrageenans is the food industry. There are also potential medical carrageenan uses for several diseases such as herpes, human papillomavirus (HPV) and HIV/AIDS [2]. Carrageenan polysaccharides are linear chains composed of repeating disaccharide units with alternating 4-linked α-D-galactopyranose (**D**-unit) or 3,6-anhydro α-D-galactopyranose (DA-unit) and 3-linked β-D-galactopyranose (**G**-unit) units with different sulfate patterns.

Materials and Methods

In this study we consider λ-carrageenan-based oligomers with two types of glycosidic linkage between the saccharide units, i.e. α(1→4) and β(1→3). In physiological conditions the O-sulfated groups are ionized and the carrageenan exists as a polyanion. However, we generally investigate here possible force-driven structural changes in neutral (non-ionized) and non-sulfated λ-carrageenan based oligomers (mono- up to pentasaccharides). The position in the oligomeric chain may also have an effect on the enforced conformational transitions. That is why the different types of dimeric (**DG** and **GD**), trimeric (**DGD** and **GDG**), and tetrameric (**DGDG** and **GDGD**) up to pentameric (**DGDGD** and **GDGDG**) structures were considered here (FIG. 1).

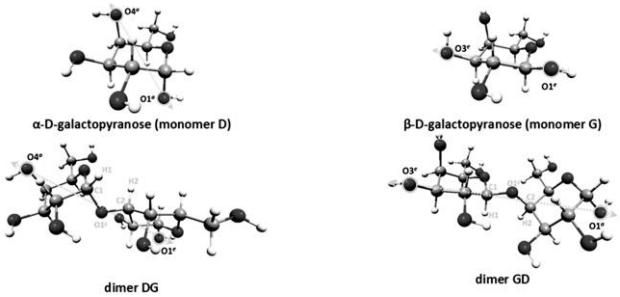

FIG. 1. he molecular systems and the stretching force modes. The superscripts a and e denote the axial and equatorial positions of the terminal –OH groups. The characteristic glycosidic torsion angles are defined as: ψ = H1C1O1gC2, φ = C1O1gC2H2.

In this study we use the Enforced Geometry Optimization (EGO) [3] method to simulate the AFM single molecule experiment. This approach involves geometry optimization in the presence of external forces acting on selected nuclei in a molecule. The optimized ground state geometries were exposed to external stretching forces, in the range from 0.00 to 0.075 a.u. usually in steps of 0.005/0.0025 a.u. (1 a.u. = 82387 pN). The external forces were applied to the terminal oxygen atoms O1 and O3/O4 depending on the structure, i.e. the potential location of a glycosidic bond(s) with another saccharide unit(s) in an oligomeric molecule (FIG. 1). The stressed structures after the EGO procedure were relaxed, i.e. re-optimized without external forces to determine whether or not the structural changes (conformational transition(s), changes in glycosidic linkage(s) etc.) induced by the stretching forces are permanent.

Results and Discussion

The EGO calculations for the discussed oligomeric structures predict several types of permanent enforced conformational transitions. The energetically preferred form in α-D-galactopyranose is of course the chair conformer $^4C_1$ and it is the starting conformation of all units in the carrageenan structures. The type of conformational conversion is determined by the location in the saccharide chain and it is a direct consequence of the different glycosidic bond types in the oligomeric structure. The results for the oligosaccharides are gathered in TABLE 1.

TABLE 1. Permanent enforced conformational transitions in the carrageenan oligomers.

Unit→	D G D G	Unit→	G D G D
	Initial conformer		Initial conformer
Oligomer↓	$^4C_1$ $^4C_1$ $^4C_1$ $^4C_1$	Oligomer↓	$^4C_1$ $^4C_1$ $^4C_1$ $^4C_1$
D	$^1C_4$	G	$^4C_1$
DG	$^1C_4$ $^4C_1$	GD	$^4C_1$ $B_{3,O}$
DGD	$^1C_4$ $^4C_1$ $^1S_3$	GDG	$^4C_1$ $B_{3,O}$ $^4C_1$
DGDG	$^1C_4$ $^4C_1$ $^4C_1$ $^4C_1$	GDGD	$^4C_1$ $B_{3,O}$ $^4C_1$ $^1S_3$

Conclusions

In order to gain a deeper understanding of the mechanism of structural changes in the non-sulfated carrageenan based oligosaccharide molecules we applied the EGO approach. In this work, four different types of permanent enforced conformational transition in the carrageenan-based oligomers were identified ($^4C_1 \rightarrow {}^1C_4$, $^4C_1 \rightarrow {}^1S_3$, $^4C_1 \rightarrow B_{3,O}$, and $^4C_1 \rightarrow {}^2S_O$). Generally, the type of the conformational conversion depends directly on the galactopyranose ring position in the oligosaccharide chain. The chair to inverted-chair enforced transition ($^4C_1 \rightarrow {}^1C_4$) is observed for the first terminal **D** units (at the non-reducing end of the chain) The internally located **D** units with the starting $^4C_1$ conformation under the external stretching forces mostly transform to the $B_{3,O}$ form. In turn, there are two potential conformational courses $^4C_1 \rightarrow {}^1S_3$ and $^4C_1 \rightarrow B_{O,3}$ for the closing terminal **D** units depending on the type (**D** or **G**) of the first oligomer unit. Additionally, the type of the terminal oxygen atoms (axially or equatorially oriented) of the oligomeric structure also determines the mechanical resistance to the external forces. These findings provide crucial information about the complicated nature of the enforced structural changes in the examined oligosaccharides.

Acknowledgments

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COLLAGEN TYPE I HYDROGELS MODIFIED WITH SODIUM ALGINATE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 51]

Introduction

Collagen is a structural protein that forms extracellular matrix of connective tissues. It is one of the most commonly used material for biomedical applications. Collagen solution may form hydrogel e.g. due to neutralization, but this process is time-consuming and the gels obtained are relatively weak [1,2].

Sodium alginate is a natural, linear polysaccharide obtained mainly from brown algae. It easily creates stiff, thermo-irreversible gels in the presence of divalent ions (Ca^{2+}) [3,4].

The aim of our work was to shorten the time of gel preparation and improve of its strength by mixing collagen solution with sodium alginate.

Materials and Methods

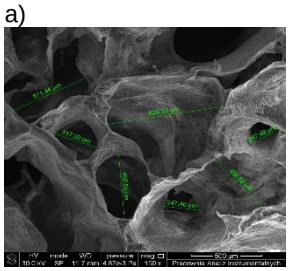
The collagen type I 0,5% solution in 0.1 M acetic acid was prepared, neutralized by 0.1M NaOH addition and mixed with 5% alginate solution at various weight ratios of dry polymers (5:5, 4:6, 3:7, 2:8, 1:9). Then the blends were dialysed against water and calcium chloride solution.

The porous structure of the lyophilized hydrogels was investigated using SEM (Quanta 3D FEG). The gels were mechanically tested (compressive strength) using Zwick&Roell Z0.5 machine. The cytotoxicity of these materials was also studied by culturing 3T3 cells with the addition of collagen/alginate hydrogel extract.

Results and Discussion

The developed method allowed to obtain rigid, homogeneous collagen/alginate hydrogels. SEM image presented in FIG. 1 shows porous structure of the materials.

a)



b)

Sample	Pore size [μm]
K5-5A	417 ± 214
K4-6A	311 ± 124
K3-7A	215 ± 87
K2-8A	205 ± 60
K1-9A	198 ± 88

FIG. 1. SEM image of porous structure of K5-5A hydrogel (a) and average pore size of various collagen/alginate hydrogels (b).

The pore size decrease when alginate content in hydrogel is bigger. Furthermore, the mechanical strength of the materials also grows (FIG. 2).

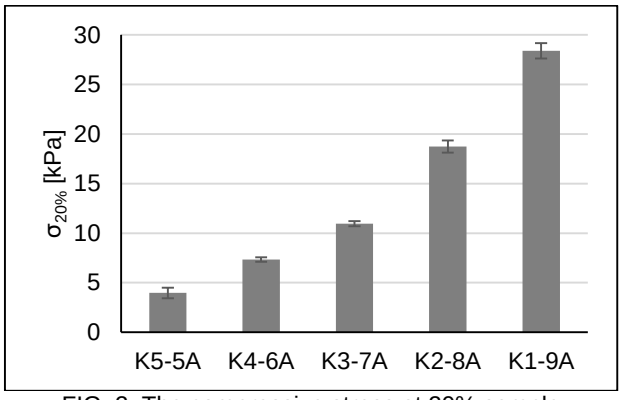


FIG. 2. The compressive stress at 20% sample deformation of collagen/alginate hydrogels.

Viability of 3T3 cells cultured with the addition of collagen/alginate hydrogel extract was also tested (FIG. 3). None of the materials shows cytotoxicity.

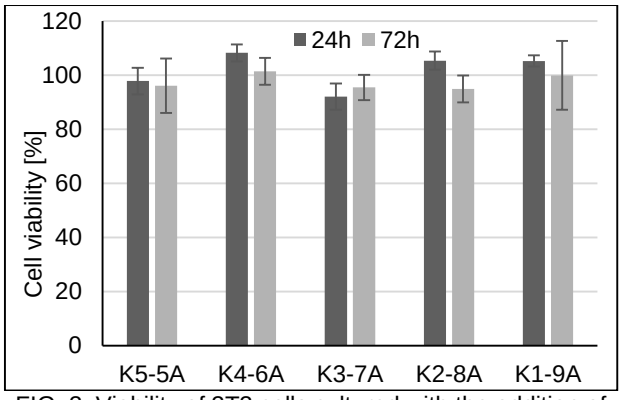


FIG. 3. Viability of 3T3 cells cultured with the addition of collagen/alginate hydrogel extract.

Conclusions

The mixing of collagen type I and sodium alginate significantly accelerate the hydrogel formation and improve its mechanical properties.

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BIOLOGICAL EVALUATION OF ZrC COATING ON STAINLESS STEEL 316L: ARE ZrC-COATINGS SUITABLE FOR CARDIO-VASCULAR APPLICATIONS?

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[ENGINEERING OF BIOMATERIALS 153 (2019) 52]

Introduction

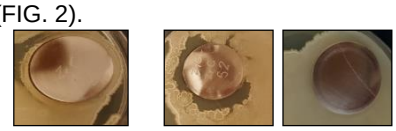
Bioreactivity in terms of the interaction of cells and tissues with the implant, occurs directly on the material surface. When using devices for cardiovascular applications, the reactions of the body to the implanted material such as protein adsorption, bacterial adhesion, phagocytosis, haemolysis, platelet activation and adhesion, and biodegradation must be considered. The healing process after implantation can be accelerated by functionalizing implant surfaces. The most known methods for surface functionalization are surface treatment with extracellular proteins (fibrinogen, collagen, albumin), the immobilization of growth factors or the treatment with the extensive cell plasma as well as whole blood. Blood as the first component of contact with the implant is crucial for solidification and healing of the implant site [1-3]. In the presented study the bacterial tests and cytotoxycity tests by using L929 were performed on the ZrC-coatings. Additionally, to evaluate the hemocompatible character of the new coatings platelets activation was executed.

Materials and Methods

ZrC-coatings were deposited on polished 316L steel substrates by using pulsed and reactive magnetron sputtering. Through spraying the Zr target in the C_2H_2 atmosphere under different flow rates, coatings with carbon content of 61 and 75 at% were deposited.
The bacteria from the American Collection of Pure Cultures Staphylococcus aureus ATCC 25922 were applied. The antimicrobial activity of the coatings was examined according to the SN 195920 standard. The susceptibility testing of the surfaces of the coatings to microbial adhesion was performed in accordance with ISO 22196: 2011.
Cytotoxicity of the specimens were analyzed by the live-dead staining assays for mouse fibroblasts (cell line L929). For the platelets activation, human peripheral blood mononuclear cells (PBMC) were isolated from the human whole blood. Platelets activation on ZrC-coatings in terms of their structure, adhesion and morphology was evaluated by scanning electron microscopy (SEM).

Results and Discussion

All coatings appeared to be bacteriostatic (FIG. 1). A low number of bacterial cells adhering to their surface was detected (FIG. 2).



61% C 75% C steel 316L
FIG. 1. Bacteriostaticity coatings.

In the present study it was found that after adsorbing of fibrinogen on the ZrC-coatings, the number of adhered bacteria increased. Probably, besides of bacterial adhesions, which in the contact with the protein initiate specific ligand-receptor processes, additionally the deficiency of coagulase or other proteins in the structure of bacteria causes them to join in those places that complement these deficiencies [4].

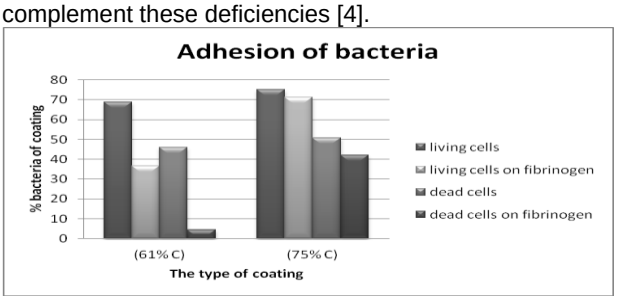
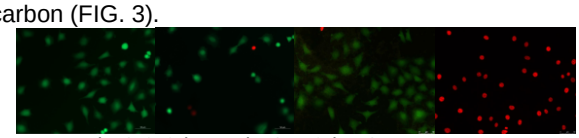


FIG. 2. Adhesion of bacteria.

Examination of the cytotoxic effects of ZrC coatings showed that only coatings with a carbon content of 61% were biocompatible with mouse fibroblasts. Cytotoxic effects were observed on samples containing 75 % of carbon (FIG. 3).



A (ZrC 61%) B (ZrC 75%) C D
FIG. 3. 24h: A, B Live/Dead Staining L929 (20x), C, D Live/Dead Staining L929 TCPS Control.

The cytocompatible coating wass selected fot platelets activation tests. The number of attached blood cells (platelets and leukocytes) on the surface is significant lower than the glass control which indicates hemocompatible character of the coating (FIG. 4). We did not observe any morphologically-destructed cells, which confirms the cytocompatible properties.

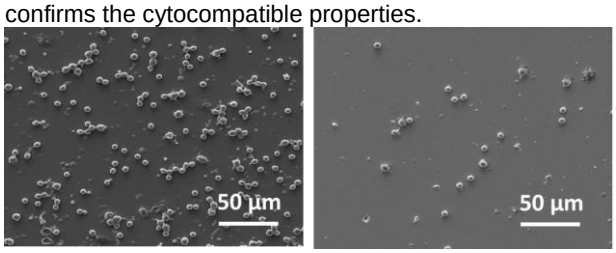


FIG. 4. SEM images of leukocytes and platelets on the lass control (left) and ZrC (right) after 4h incubation.

Conclusions

The study of ZrC-coatings showed that their use in cardiovascular applications can be considered in the future. The ZrC-coatings containing 61 % of carbon content showed the favourable biological properties. These coatings showed cytocompatible character to mouse fibroblasts and did not affect platelets activation, which indicates their hemocompatible properties. Such coatings could improve cardiovascular devises in terms of their stability, anticorrosion character, bactericidal properties and enhanced hemocompatibility.

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INFLUENCE OF PLASMA-DEPOSITED COATING OF SILVER NANOPARTICLES EMBEDDED IN A SiOC:H MATRIX ON THE ADSORPTION OF FIBRONECTIN

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[ENGINEERING OF BIOMATERIALS 153 (2019) 53]

Introduction

At any given time, out of 100 hospitalized patients, 7 in developed and 10 in developing countries will acquire at least one health care-associated infection (HCAI). The development of HCAIs is mainly due to the material's surface colonization by microorganisms. Adherent bacteria organize themselves to form a biofilm, once the extracellular polymeric matrix made of proteins, polysaccharides and lipids is formed [1]. The biofilm structure renders the bacteria highly resistant to existing antibacterial agents as antibiotics, and accordingly, there is a growing need to develop new strategies to inhibit this bacterial structuration. This project takes advantages of the known antibacterial potential of silver nanoparticles (AgNPs) [2]. However, to achieve a long-lasting delivery of Ag^+ ions, a fast oxidation of the AgNPs should be avoided. Protection of the AgNPs was ensured through coating with a plasma-deposited organosilicon matrix [3,4]. This study will focus on the adsorption of fibronectin (FN) on plasma-deposited organosilicon (SiOC:H) surfaces containing AgNPs, as protein adsorption is the first step towards bacterial biofilm formation.

Materials and Methods

An axially-asymmetric radiofrequency capacitive plasma process was used to deposit a SiOC:H matrix and AgNPs on quartz substrates. The matrix was obtained using a gas mixture of hexamethyldisiloxane (HMDSO) and argon, while the AgNPs were synthesized by sputtering of the upper silver electrode in an argon plasma [3,4]. Optical Emission Spectroscopy (OES) was used to control the plasma deposition process. A droplet of 10 µL of human FN solution in HEPES buffer at a concentration of 200 µg/mL, was deposited on the plasma-deposited SiOC:H surfaces with or without AgNPs. The adsorption lasted for 1h and was followed by 3 successive washings in nanopure water with vortex and drying. Atomic Force Microscopy (AFM), X-ray Photoelectron Spectroscopy (XPS) and dynamic contact angle measurements were used to characterize the surfaces and the FN conformation after adsorption.

Results and Discussion

OES spectra revealed the presence of Ag during the plasma deposition of the SiOC:H containing AgNPs (data not shown) while no Ag was detected for the matrix alone. XPS spectra (TABLE 1) clearly put in evidence the presence of Ag in the samples containing AgNPs. In addition, the apparition of N1s feature, characteristic of proteins, showed the presence of adsorbed FN while no N1s peak was detected on the fresh plasma-deposited SiOC:H and SiOC:H+AgNPs thin films. The FN adsorption is further confirmed through the decrease of %Si and %Ag concentration with the amount of adsorbed FN on the surfaces. Moreover, the difference of %N between the FN/SiOC:H and FN/SiOC:H+AgNPs suggested either a modification in the FN organization or in quantity of adsorbed FN influenced by the AgNPs. In fact, the first hypothesis is that the proteins could adopt an organization which can hide or show some functional groups depending on the surface where they adsorb. The depth of penetration of XPS being only of 5 nm and the FN size of 70 nm, these functional groups could be more or less detected. The second hypothesis is that the AgNPs have an effect on the amount of adsorbed FN due to some interactions.

TABLE 1. Elemental composition obtained by XPS survey.

Sample	Elemental composition (%)				
	O	Si	C	Ag	N
SiOC:H	36.9	16.1	46.4	-	-
FN/SiOC:H	20.6	7.2	61.2	-	11.0
SiOC:H+AgNPs	30.4	14.6	45.7	3.4	-
FN/SiOC:H+AgNPs	20.1	4.5	62.9	2.8	9.6

AFM images also confirmed the presence of adsorbed FN by modification of the roughness (FIG. 1). Moreover, the difference of roughness between FN/SiOC:H (2) and FN/SiOC:H+AgNPs (4) showed that the AgNPs have an influence on the FN adsorption leading to a conformation modification of the proteins when they are brought in contact with the surface.

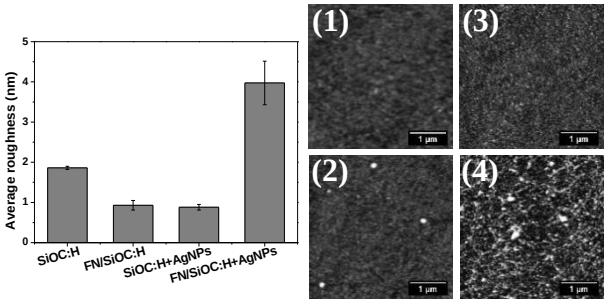

FIG. 1. Average roughness and AFM images (4 µm x 4 µm) of: (1) SiOC:H, (2) FN/SiOC:H, (3) SiOC:H+AgNPs and (4) FN/SiOC:H+AgNPs.

Conclusions

XPS spectra and AFM images confirmed the presence of Ag and adsorbed FN on the surfaces. These analyses also suggested a modification of the protein conformation or in the quantity of proteins after adsorption on the surface of thin organosilicon films containing AgNPs. In addition, FTIR studies will be performed to evaluate the secondary structure of the adsorbed protein. Finally, ELISA tests will be used to identify the availability of the specific sites (RGD) of the protein.

Acknowledgments

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ANTIMICROBIAL PROPERTIES OF POLYMERS USED IN 3D PRINTING METHODS

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Introduction

The possibilities of 3D printers are more and more often used for the production of medical devices, personalized prostheses as well as materials and elements of devices that support a sick person. The condition that allows the use of 3D printing is its non-toxicity in relation to the human body, bacteriostaticity, insensitivity to environmental factors and resistance to abrasion. Commonly used materials used in this printing technique are PLA (polylactide - poly (lactic acid) and ABS (poly acrylonitrile-co-butadiene-co-styrene). ABS and PLA belong to thermoplastic materials that are easy to machine, unfortunately May too low strength parameters, hence chemical modification is necessary PLA belongs to the group of aliphatic polyesters Its advantages are: biodegradability, odorlessness, high resistance to UV radiation and extraction from natural resources, that is environmentally friendly ABS has good plasticity And solubility in organic compounds, thanks to which you can easily combine printed parts [1,2].
The present study evaluated the bacteriostaticity of the polymers mentioned above for 3D printing, PLA (polylactide) and ABS (acrylonitrile butadiene styrene terpolymer) and their microstructural properties using XRD.

Materials and Methods

Four species of microorganisms from the American Collection of Pure Cultures were tested: Actinomyces viscosus ATCC 15987 + D1291, Escherichia coli NCTC 12241 / ATCC 25922, Staphylococcus aureus NCTC 12981 / ATCC® 25923, Streptococcus sanguis ATCC10556. In the study, polymers used for 3D printer - PLA (yellow discs) and ABS (red discs) were tested.
The antimicrobial activity of the polymers was evaluated by the direct method based on the criteria contained in the SN 195920 standard. The bacterial culture was performed on TSA medium.
The susceptibility of the surfaces of the coatings to microbial adhesion was carried out in accordance with the procedures included in the standard: ISO 22196: 2011 (Plastics: Measurement of antimicrobial activity of plastics and other non-porous surfaces)
with modifications regarding the assessment of microbial viability.
The microstructure of the test specimens was investigated by Empyrean's XRD-Expert Multiprocesore Diffractometer.

Results and Discussion

Bacteriostatic assessment of coatings showed in each case tested bacteria their inhibition (FIG. 1).

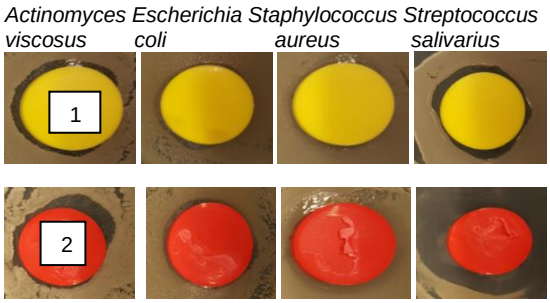

FIG. 1. Antimicrobial activity of polymers.

In the assessment of bacterial adhesion to polymers (FIG. 2), it was observed that the least live and dead bacteria were on the PLA polymer samples in the case of *Streptococcus sanguis*. Actinomyces viscosus bacteria on the ABS polymer were found the most. Assessment of adhesion to bacterial polymers showed that the number of bacteria on the ABS material is definitely higher.

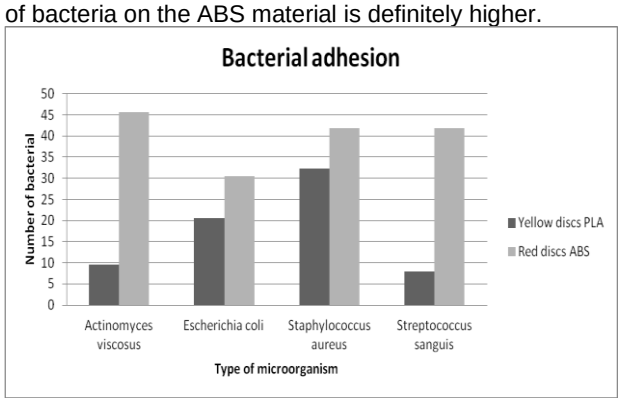

FIG. 2. Number of cells that adhere to polymers.

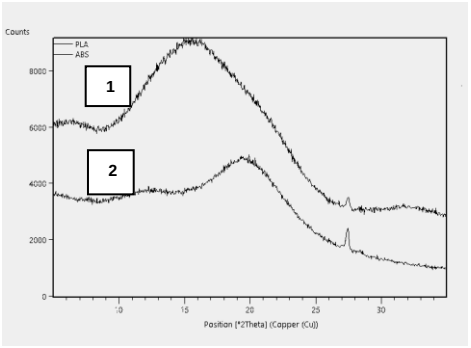

FIG. 3. XRD diffraction of 1-PLA, 2-ABS. CoKα1 (λ = 0,17902 nm)

Microstructural studies (XRD) of polymeric samples (FIG. 3) have shown that they are characterized by crystalline - amorphous submicroscopic structure, confirming their ability to develop crystalline areas.
On the diffractogram of the ABS polymer sample, visible diffraction lines from planes (002) are visible. In the case of PLA polymer samples, a diffraction line from planes (200) [3] is visible.

Conclusions

The tested materials showed static action on bacteria, hence their use in places with a special threat of microbial contamination can be considered.

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UTILIZATION OF GRAPHENE-BASED MATERIALS AND MESENCHYMAL STEM CELLS IN REGENERATIVE MEDICINE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 55]

Introduction
One of the challenge of regenerative medicine is to obtain a native and functional cartilage tissue *in vitro*, that can be used for articular cartilage repair. Recent evidence indicate innovative approaches that utilized stem cells (SCs) and biomaterials investigations as one of the promising tool that may be used in biomedical applications. Mesenchymal stem cells (MSCs) possess huge proliferative capacity, high paracrine activity and low immunogenicity. Moreover, MSCs can be differentiated into cells of mesodermal origin. There is also evidence that physical stimulation of the differentiation process is very important, but it is still not well known. Thus, in this study, we tested MSCs together with graphene-based materials, that possess unique physical, chemical, and biological properties to improve the effectiveness of chondrogenic differentiation of MSCs.

Materials and Methods
GO-based materials were prepared according to the Marcano method and then were modified with different types of metal nanoparticles (ITME Institute). Next, GO materials were sterilized and utilized as culture surface dedicated for human umbilical cord Wharton's jelly mesenchymal stem cells (hUC-MSCs). The influence of GO-based materials on biological properties of hUC-MSCs was evaluated. Proliferation test was performed by Scepter 2.0 Automated Cell Counter (Merck Millipore). Cell viability was measured by LSR Fortessa flow cytometer by FITC Annexin V Apoptosis Detection Kit (BD Biosciences). Moreover, the influence of GO on induction of chondrogenic differentiation process of hUC-MSCs was studied by gene expression (real-time PCR; Life Technologies) and protein level analysis (alcian blue staining; Sigma Aldrich).

Results and Discussion
The results revealed that graphene-based substrates constitute non-toxic surfaces for culture of hUC-MSCs. We observed, that GO may slightly modulate proliferation and survival of hUC-MSCs. Moreover, the obtained results indicate a positive effect of analyzed graphene-based surfaces on the induction of chondrogenic differentiation of hUC-MSCs. Interestingly, we revealed, that modification of GO with metal nanoparticles induce the positive effect of this process. We observed a significant increase in the number of colonies containing proteoglycans (e.g. aggrecan) that are formed on native and modified GO-based substrates. In addition, we observed an increased size and rate of formation of microbodies during the experiment. Moreover, the results of the gene expression analysis indicate the stimulation of the chondrogenic differentiation process in hUC-MSCs cultured on both GO scaffolds: native and modified. Quantitative analysis demonstrated an increase level of the transcripts of genes associated with chondrogenesis (e.g. SOX9 and COL2A1).

Conclusions
Performed studies provide evidence that tested graphene-based substrates (native and modified) constitute a suitable surface for hUC-MSCs propagation and may induce differentiation of MSCs towards cartilage cells. However, further studies are required to optimize new protocols for cell preparation for orthopedic applications in the future.

Acknowledgments
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ISOLATION AND CULTURE OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN GMP QUALITY CONDITIONS FOR ITS FURTHER APPLICATION IN TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 56]

Introduction
In recent years, the clinical application of stem cells in tissue engineering and regeneration including the treatment of bone and cartilage defects is becoming more significant. Adipose tissue (AT) represents an abundant and accessible source of stem cells including mesenchymal stem / stromal cells (MSCs) and may be a starting material for isolation of AT-derived MSCs (AT-MSCs). MSCs can differentiate into multiple tissues including bone, cartilage etc. It has been demonstrated that upon intra-articular administration into injured joint, MSCs induce cartilage replacement and were detected in the newly formed tissues. However, growing evidence has recently indicated that the paracrine activity of the transplanted MSCs might be predominantly responsible for stimulating pro-regenerative effects in a place of transplantation including the cartilage repair. For clinical applications, AT-MSCs must be manufactured in accordance with Good Manufacturing Practice (GMP). Directly prior to intra-articular administration, AT-MSCs can be mixed with some biopolymers which can serve as a carrier creating 3D environment mimicking stem cell niches.

Materials and Methods
The human adipose tissue were collected during liposuction procedure from subcutaneous area of human donors. The procedure of AT-MSCs isolation and culture were optimized during a series of conducted experiments. All materials and reagents used for manufacturing of AT-MSCs were sterile and GMP quality or of equivalent standard. Morphology of adherent cells were analyzed by phase-contrast microscopy. Identification of these cells were carry out in accordance with the International Society for Cellular Therapy position statement paper. To evaluate potential application of AT-MSCs in regeneration of damaged cartilage, cells were differentiated into chondrocyte under high pressure (2 PSI, 5 PSI) and low oxygen (5%) conditions using Avatar Cell Control System. In the last step, we designed the composition of a carrier solution for AT-MSCs enhancing viability and functionality of these cells before and after application into site of injury.

Results and Discussion
Based on our translational research we selected the optimal condition for AT-MSCs isolation and their propagation *in vitro* using materials and reagents compliant with GMP. The cultured cells were confirmed as MSCs based on appropriate identity and phenotype assessed accordingly to specific criteria provided by the International Society for Cellular Therapy. We have shown that isolated and expanded adherent fraction of AT-MSCs maintained in standard culture conditions, express following antigens: CD44, CD73, CD90, CD105 (characterising MSCs) and do not possess CD45, CD34, CD14, CD19, HLA-DR antigens on their surface. Moreover, we have also demonstrated their capacity for trilineage mesenchymal differentiation (to osteoblasts, chondroblasts/ chondrocytes and adipocytes) *in vitro*. Moreover, the potential to differentiate into chondroblasts/chondrocytes was also confirmed in microenvironment resembling conditions in joints including appropriate oxygen concentration and pressure by employing a novel platform for cell culture such as *Avatar System* allowing for tuneable control of oxygen level and hydrostatic pressure (1-21% O_2 and 1-5 PSI, respectively). Our data suggest that AT-MSCs differentiate into chondrocyte-like cells in standard culture conditions (21% O_2; 1 PSI) and in the microenvironment resembling joint niche (5% O_2, 2 PSI). In the next step, we developed the optimal composition of medium for preparing of cell suspension for injection, which allows maintaining high viability of AT-MSCs before and after application into site of injury.

Conclusions
We successfully optimized efficient protocol for isolation and culture of AT-MSCs using GMP- grade reagents and materials. The optimized procedures for validation of the manufacturing process in GMP facility have been also established. During the validation of the manufacturing process accordingly to these procedures, the safety profile of the product will be assessed by analyses of genotypic stability, tumorigenicity, phenotypic profile and multipotent differentiation potential.
AT-MSCs due to their ability to differentiate into chondrocytes and secretion of wide range of bioactive factors can be effectively use in tissue engineering. AT-MSCs exhibit properties for “regenerating, repairing or replacing a human tissue” and can be classifies as “tissue engineered product” (According to Reg. 1394/2007).

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CALCIUM ALGINATE-BASED ANTIBACTERIAL FILMS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 57]

Introduction

Sodium alginate (AL) is a salt of alginic acid, a linear polysaccharide composed of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid residues. Due to its gelling properties, biocompatibility, non-toxicity, biodegradability, sodium alginate is the most extensively studied material for the preparation of polymeric films. Such films have a wide variety of applications mainly in the food, cosmetic, medical and pharmaceutical industries [1]. Glycerol, sorbitol, xylitol, polyethylene glycol, or mixtures thereof are used as plasticizers of alginate films [2].

Various essential oils are used in order to prepare films with antibacterial activity. Namely, garlic, elicriso italic, lavender, eucalyptus, oregano, rosemary, chamomile blue, lemon, peppermint, cinnamon and lemongrass are incorporated in the films as active substances [3].

This study aimed to develop calcium alginate-based flexible films covered with gum of Chios mastic for the prevention of bacterial growth.

Materials and Methods

Two types of films were prepared and modified with antibacterial material. The first group of samples were prepared by dissolving AL powder in glycerol-water solution (5 wt.% glycerol) to yield a 3 wt.% AL solution. The second group of samples were prepared by dissolving hyaluronic acid (HA) in glycerol-water solution (5 wt.% glycerol) to yield a 0,5 wt.% HA solution. Further, AL powder was added into prepared solution to reach a concentration of 3 wt.%. After complete solubilisation both solutions were poured into Petri plates (30 mL/plate with a diameter of about 90 mm) and sprayed with 1% calcium lactate solution. After 10 minutes, the spraying was repeated and the plates were placed in a Binder ED53 (Binder, Germany) oven at 37°C for 72 hrs. The antibacterial properties were provided by immersing the films in a 5 wt.% solution of mastic gum in ethanol. The thickness of the films was measured with a micrometer (293 MDC-MX, Mitutoyo Co., Kawasaki, Japan). The MRS 120-3 moisture analyzer (Kern, Germany) was used to measure moisture content. The mechanical properties were determined using a universal material testing machine Zwick/Roell BDO-FB O.5 TH (Zwick, GmbH & Co, Ulm, Germany). The zone of inhibition assay on solid media was used for the evaluation of the antibacterial effect of films against Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus and Staphylococcus epidermidis*) bacteria.

Results and Discussion

Two types of flexible films were prepared: calcium alginate (Ca-AL) and its composite film with hyaluronic acid (Ca-AL-HA). The antibacterial properties of the films were ensured by immersing the films in a solution of Chios mastic gum in ethanol.

Surface coating had a direct effect on the characteristics of the films (TABLE 1). Antibacterial layer formation caused a decrease in the thickness of the films. It was found that such differences in the thickness of the films were affected by the solvent. The obtained films were swollen in water. However, the use of ethanol solution slightly shrank the films and caused a decrease in thickness.

TABLE 1. Characteristics of the films.

Film No.	Film type	Film thickness, μm ± SD	Moisture content, % ± SD
1	Ca-AL	260 ± 30	10 ± 0,9
2	Coated Ca-AL	230 ± 10	2 ± 0,2
3	Ca-AL-HA	310 ± 40	11 ± 1,2
4	Coated Ca-AL-HA	240 ± 20	3 ± 0,4

The moisture content of the films depended on the film composition. The moisture content was larger in Ca-AL-HA films due to HA ability to retain water.

The mechanical properties of formed films were investigated. The results showed that the addition of glycerol and HA into the composition of the films caused a significant reduction in the rigidity.

Films (1 cm^2 in size) coated with mastic gum were used to investigate the antibacterial properties against Gram-negative and Gram-positive bacteria. Uncoated Ca-AL, Ca-AL-HA films were studied for comparison. As expected, uncoated Ca-AL film was not effective against any test microorganisms. Uncoated Ca-AL-HA and Ca-AL coated with Chios mastic gum showed similar antimicrobial activity against *Escherichia coli*. Results showed, that the strongest antibacterial activity had Ca-AL films with HA and Chios mastic gum layer. The clear zones of inhibition directly underneath the film pieces were observed in the plates inoculated with *Escherichia coli* and *Staphylococcus aureus*. Also, a weak inhibitory effect against *Staphylococcus epidermidis*, indicated by minimal growth underneath film pieces were observed. However, *Pseudomonas aeruginosa* was resistance to all tested film compositions.

Conclusions

The observed results confirm that the preparation of calcium alginate flexible films with antibacterial properties is a complex process. The addition of plasticizer clearly improved the flexibility of the films.

The presence of HA and Chios mastic gum layer on the originally prepared films improved antibacterial properties. The films were effective against Gram-negative and Gram-positive bacteria.

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POLYURETHANE COMPOSITES AS A POTENTIAL 3D SCAFFOLD FOR MESENCHYMAL STEM CELLS IN CARTILAGE REGENERATION

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[Engineering of Biomaterials 153 (2019) 58]

Introduction

Osteoarthritis (OA) is characterized as a degeneration of articular cartilage. Cartilage is a highly specialised tissue with a low regenerative potential. Traditional OA treatment methods are not able to stop the disease progression and are mainly focused on pain control. Therefore there is a growing interest in regenerative medicine such as cell therapy, where cells are injected directly into the damage tissues, and tissue engineering, where cell-scaffold combinations are used to repair the tissues [1, 2]. One of the most promising type of stem cells in cartilage regeneration are mesenchymal stem cells (MSCs) that have the ability to differentiate into chondrocytes [2].
In our research we tested the possibility of using modified polyurethane composites as a biocompatible 3D scaffold for Mesenchymal Stem Cells.

Materials and Methods

In the studies were used native and modified polyurethane composites synthesized by the Project partner (AGH). The experimental scheme was planned according to the guidelines of the ISO 10993 Biological evaluation of medical devices.
The effect of PU composites on human umbilical cord MSCs was checked by indirect and direct tests. Indirect tests were to examine the effect of substance released from the polyurethane on the MSCs. For indirect tests were used Cell Counting Kit 8 (Sigma Aldrich), (CyQuant Cell Proliferation Assay Thermo Fisher Scientific) and Cytotoxicity Detection Kit (Sigma Aldrich). Direct tests were performed to check the effect of cell composite interaction on the proliferation of MSCs. For indirect test was used ATPlite Luminescence Assay (Perkin Elmer).
Each test was performed according to the manufacturer's protocol in three independent replicates.

Results and Discussion

Our results obtained in short term indirect tests show that the cell viability and proliferative capacity of mesenchymal stem cells exposed to soluble substances released from the tested polyurethane composites are comparable to the control conditions. There was also no cytotoxic effect observed. This may indicate that the investigated polyurethane composites do not secrete toxic substances for MSCs. In tests of direct contact of MSCs with the tested biomaterials a significant decrease in cell viability was observed, followed by a slow increase after 72 hours. This could have been influenced by the change in cell culture method (from 2D to 3D) and the composition of the culture surface (from hydrophilic to more hydrophobic) relative to standard control conditions. In addition, the lack of a cell proliferation assay dedicated to porous and non-transparent 3D materials affects the correct analysis of interaction of the investigated materials with the cells.

Conclusions

The presented results indicate the need for further research for a detailed examination of the impact of native and modified polyurethane composites on the biological properties of mesenchymal stem cells.

Acknowledgments

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BIO-BASED POLYURETHANE FOR SURGICAL APPLICATIONS – INFLUENCE OF PEG MOLAR MASS, BIOLOGICAL AND THERMAL PROPERTIES OF POLYURETHANE COMPOSITES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 59]

Introduction

History of medicine shows that due to the constant development of modern operational techniques, as well as an increasingly broad range of available biomaterials interest in using of synthetic materials in surgical is constantly growing. Polymeric materials, due to their unique properties, are quickly replacing other biomaterials, such as ceramics or metals and theirs alloys in medical applications. Polyurethanes (PURs) are a group of polymers with interesting properties and the broadest range of applications. The main substrates for the preparation of PURs are isocyanate, chain extender and polyol. Isocyanate and chain extender create in the PUR so-called rigid segment while the polyol – flexible segment [1]. So far, Poly(ε-caprolactone) (PCL) - based PURs has been most widely studied because the PCL soft segment is characterized by a high modulus and also ultimate tensile stress. PCL is a biocompatible, semi-crystalline and biodegradable aliphatic polyester having promising thermal properties [2,3]. It is degradable enzymatically and hydrolytically. The biodegradability and mechanical properties of PCL-PUR can be tailored for various applications by varying the chemistry or molecular weight of its components [2].

Materials and Methods

Poly(ε-caprolactone) diol with a 2000 average molar mass was used as a soft segment. 1,6-hexamethylene diisocyanate (HDI) was used in stoichiometric amounts. Dibutyltin dilaurate (DBTDL) was used as a catalyst. PURs were synthesized with starch (SA) and 1,4-butanediol (BD) as a chain extender. In order to improve the mechanical properties and bioactivity of the biomaterial, hydroxyapatite (HAp) was applied, while poly(ethylene glycol) (PEG) with a 4000, 6000, 8000 and 10000 average molar mass was used as phase change material (PCM) for storing curing thermal energy. PUR biomaterials were obtained using a two-step bulk polymerization method. PCL, SA, BD, PEG and HAp were dried before synthesis, while the other reagents were used as supplied. PEG was introduced into a flask, HDI and DBTDL were added and stirred under nitrogen to obtain the pre-polymer. The BD, SA, PEG and HAp were dispersed by sonication. In the second stage, a chain extension process was performed. After thorough mixing of ingredients at RT, the samples PUR were cured at 80°C for 24 h [4]. Differential scanning calorimetry (DSC), scanning electron microscopy (SEM), thermogravimetry (TG), dynamic mechanical analysis (DMA) and Fourier transform infrared spectroscopy (FT-IR) techniques were used for characterization of the obtained PURs.

Results and Discussion

The thermal degradation of PUR is characterised by the decomposition of urethane bonds and the degradation of soft segments as well as the evolution of volatile components. Thermogravimetric analysis TG/DTG of biodegradable polyurethanes before (FIG. 1) and after incubation in a solution of phosphate buffered saline (PBS) at 37°C show that incubation changes degradation pathways of PUR.

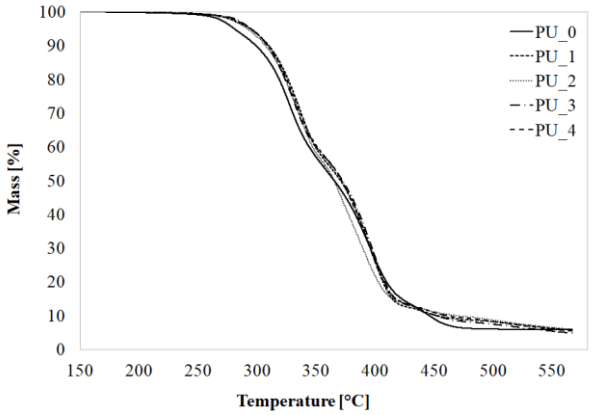

FIG. 1. TG profiles of PU samples.

The SEM images indicate that the surface of the PURs after exposure in PBS is defected. The SEM images and EDS analysis confirmed the incorporation of hydroxyapatite and starch components into the PUR matrix. The structure of PURs was confirmed by FTIR method. The absorption bands from –OH groups in at 3570 cm-¹ confirms that HAp was chemical bonded to PUR chains.

Conclusions

In this work polyurethane modified with PCL, poly(ethylene glycol) as PCM and hydroxyapatite were obtained using a two-step bulk polymerization method. The structure of the synthesized PURs was confirmed by FTIR technique. The mechanical properties of biodegradable PUR with PEG were better in comparison to the polyurethanes without PEG. The degradation of polyurethanes with PEG at the temperature of 37°C in a solution of phosphate buffered saline (PBS) seems to be faster, what was confirmed by TG method and SEM observations.

Acknowledgments

Authors are grateful to the Polish National Science Centre for financial support under the Contract No. UMO-2016/22/E/ST8/00048.

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BIORESORBABLE PERIPHERAL VASCULAR STENT WITH SHAPE MEMORY EFFECT

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[ENGINEERING OF BIOMATERIALS 153 (2019) 60]

Introduction

Peripheral Arterial Disease (PAD) is primarily affected by the deposition of fat and calcification in the Legs arteries and is called atherosclerosis. PAD more common in the legs than the arms. Approximately 8.5 million people in the United States have PAD. General population awareness of PAD is estimated at 25%, based on prior studies [1]. The treatment of this illness mostly is a peripheral angioplasty which is a low invasive procedure. Under local anesthesia the fatty plaque or blockage is pressed against the peripheral artery walls using balloon or in some case balloon with stent. Nowadays, the peripheral stents are mostly NiTi alloys made which may affect the problems for patients because these stents are no degradable and stay with the patient for whole life. Any accident which may cause uncontrolled press in implanted stent area provides serious patient life risk.
Therefore the bioresorbable peripheral vascular stents have potential in the treatment of PAD.
The present result contains the part of work on manufacturing bioresorbable peripheral vascular stents with shape memory effect [2].

Materials and Methods

The vascular stents were prepared via microinjection of terpolymer lactide based using the MicroPower (battenfeld) mould injector

Results and Discussion

The stents manufacturing parameters allow to produce well repeatable shape. The primary set of MicroPower (battenfeld) equipment has possibility to inject polymers only in one stage of mould cooling. The hot material goes to heating mould(temperature of mould was near Tg glass transition of polymer). The elements were released easily from mould but high shrinkage of material generates deformation in key point of stent structure (FIG. 1A), which affect in shape crimped stent on balloon (FIG. 1B). This effect was observed because the stream of material in mould was cooled down too fast and reach temperature of shape memory transition in situation when mould was being filled. The problem could be solved in two way: preparation of post-processing procedure or change the system of mould cooling. Both methods were checked. During post-processing conditioning of stents in special grips were held the stent shape in correct position (FIG. 1B and FIG. 2A) but the material morphology was changed. The DSC thermal curve of conditioned stents revealed increase of crystallinity. These conditioned stents were also determined at in vivo test (the pig was used to this investigations). The results of it are presented in FIG. 2. B. shows the implanted stents behaviour after highly strength spasm of pig vascular and (down position of picture in FIG. 2B) shape stent recovery (temperature of shape transition similar to the body temperature).

Summarized of this experiment, the selected conditioning parameters (post-processing) allow to obtain stents with good working shape memory effect which was proofed at in vivo tests. The second way of solvatation of the problem of shrinkage is remodelling of set equipment (MicroPower Battenfeld) which was done and the experiment is still going with satisfying results of preliminary experiments.

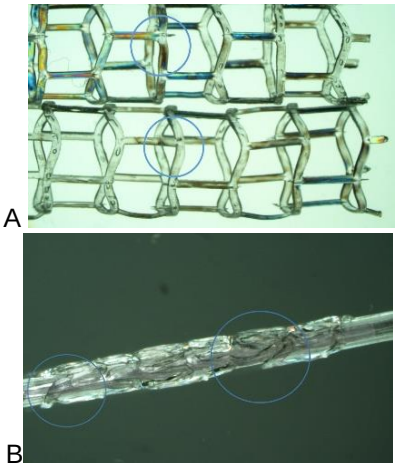

FIG. 1. A) stent with shrinkage effect and stent after post process conditioning, B) crimped shrinkage stent on catheter balloon with observed not regular deformation.

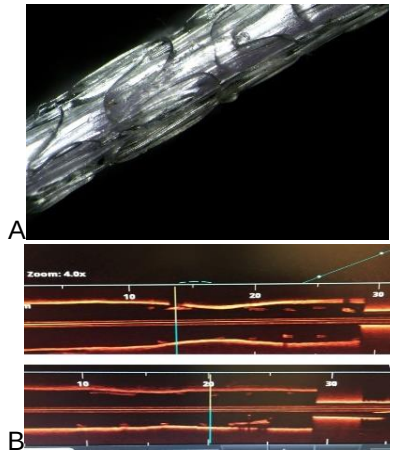

FIG. 2. A) crimped stent on catheter balloon with regular correct deformation, B) optical coherent tomography (OCT) image of a longitudinal section of pig leg vascular with stent during self-opening. (UP position) start opening, (down) full opened.

Conclusions

Presented results show next step forward to solve problem in PAD, nevertheless the production whole range of diameters/ lengths stents is unfortunately out of mould injector hardware limits.

Acknowledgments

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LONG TERM LOCAL EFFECT AFTER IMPLANTATION AND TOXICITY OF MODIFIED Ti6Al7Nb ALLOY SURFACE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 61]

Introduction

A further long-term biological study of ReligaHeart® ROT [1] device material construction was performed in order to allow the device for clinical utilisation in long-term heart support. Modification of the well-known glow discharge assisted nitriding process called as active screen plasma nitriding has been used to produce of TiN+Ti_2N+αTi(N) diffusive surface layers, improving the mechanical as well as short-term biocompatible properties of the titanium alloy [2,3].

As the final biological material evaluation, the long term local effect after implantation of the modified TiN surface as well as ceramic composite ZrO_2-Y_2O_3 was tested in vivo using small animal model, to complete the complex biocompatibility assessment required by ISO 10993 standard.

Materials and Methods

The athrombogenic diffusive nitrided surface layers TiN+Ti_2N+αTi(N)- type have been produced on Ti6Al7Nb titanium alloy surface, with the roughness of Ra=80nm, using plasma nitriding process with active screen. Biomaterial flat samples of titanium (TiAl67Nb) and titanium with nitrid layer (TiN+Ti_2N+αTi(N)) were in form of discs,14mm diameter and ZrO_2-Y_2O_3 8mm diameter and 1,5mm thickness were sterilized with ETO as the final device RH ROT sterilization method (EOGas 4, H.W.Andersen Products Ltd.).

The in vivo investigation was performed according to biocompatibility standard for medical devices, including tests for local effects after implantation (ISO 10993-6) and systemic toxicity (ISO 10993-11).

Local effects after implantation and systemic toxicity tests were carried out with the utilization of New Zealand white rabbits (n=40), both sexes, weighing over 2kg. 10 animals were used for each biomaterial subcutaneous implantation (4 implants for the titanium alloy and titanium alloy with TiN surface layer group, 2 implants in the group with zirconia implants) and 10 animals as control group (only surgical procedure, no biomaterial implanted).

Biomaterials dosage were calculated in order to select the proper sample mass comparing to animal mass, to simulate number of biomaterial kg used in blood pump recalculated for 1 kg of human body. After 4 and 12 weeks, the final observation period was 26 weeks. Every day the post-operative scar macroscopic evaluation was carried out (healing level, tissue status around the implant location). General animal behavior and condition were observed. Before the implantation as well as before euthanasia the blood was collected for hematological and biochemical evaluation and the animals were weight. The macroscopic evaluation of post-operative scar and tissues around biomaterial implants were done. After the experiment vitals samples (heart, thymus, liver, spleen, kidneys and lungs) were collected for histopathological examination.

Results and Discussion

Standardized tests to local tissue reaction and systemic toxicity, demonstrated the safety and biocompatibility of evaluated materials: titanium, titanium with nitride layer and zirconium, after 26 weeks post-implantation. Blood samples assessment did not revelled a negative impact of the investigated materials on tested animals. The testes parameters remained at a constant level. Clinical observation of animals showed no abnormal behaviour. The post-operative wound healed without complication. Finally the histopathological evaluation of the implantation area (FIG. 1) and internal organs reveled no normal healing process around the implants and changes in the internal organ structure.

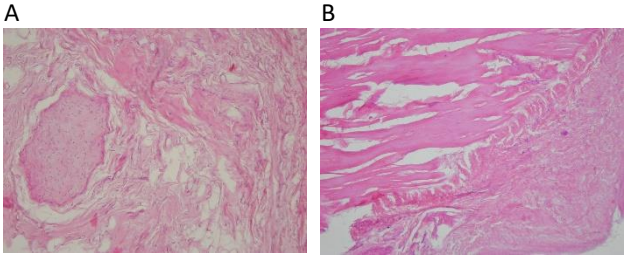

FIG. 1. Histopathological analysis of the implantation area. (A) titanium group (b) titanium with nitride layer group. No changes were found.

Conclusions

The performed studies showed in the long-term evaluation proper local effects and no syndromes of systemic toxicity of the nitrided layers TiN+Ti_2N+αTi(N)-type produced on Ti6Al7Nb titanium alloy surface, using plasma nitriding process with active screen. The results together with in-vitro biocompatibility evaluation as well as short term in-vivo studies carried out before, confirmed that the biomaterial can be safety used in the construction of implant having contact with blood, especially the Polish implantable rotary blood pump.

Acknowledgments

This work has been financially supported by the Polish Artificial Heart project and by the National Centre of Research and Development - Project no.STRATEGMED-2/ RH-ROT/266798/15/NCBR/2015.

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MECHANICAL AND BIOLOGICAL ASSESSMENT OF CARBON FIBER-REINFORCED PEEK COMPOSITE MATERIALS INTENDED FOR LARYNGEAL PROSTHESES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 62]

Introduction

The Larynx cancer is the most common squamous cell carcinoma in the head and neck area (Head and Neck Squamous Cell Carcinoma - HNSCC). It is the seventh most frequently occurring malignant tumor in the male population in Poland [1]. In the treatment of patients with high clinical advance of larynx cancer, the most common procedure is total laryngectomy. It is a mutilating procedure, as a result of which the larynx is completely removed. A patient with laryngectomy is deprived the basic organ involved in creating the voice. For many years, voice prostheses have been used to rehabilitate the speech of laryngectomized patients. The implantation of such a prosthesis involves the creation of a fistula communicating the trachea with the esophagus, which enables the development of tracheo-esophageal speech. Within the obtained fistula a voice prosthesis is placed. In Poland, the most commonly used silicone prostheses are Provox Atos Medical AB, Hörby, Sweden) and Blom-Singer (InHealth Technologies, Carpinteria, CA, USA). According to literature data, the average pot life of an implanted prosthesis is 3-6 months [2,3,4]. The objective of the study was to manufacture and assess the mechanical durability under dynamic loading conditions and biological behavior of composites manufactured from PEEK and selected carbon fibrous reinforcements. In our experiments, such composites are to be used as materials for voice prostheses.

Materials and Methods

The following components were used to manufacture the composite samples consisting of carbon fiber and PEEK polymer: Polyether ether ketone (PEEK 150PF,) delivered by Victrex was the matrix, (2D) carbon fiber cloths delivered from Porcher Industries Composites, code named Pi preg® 3106-P17, multiwalled carbon nanotubes (CNT) provided by NanoAmor, USA. The nanotubes had diameters in the range of 10–30 nm and were 1–2 μm long. The PEEK/CF composites manufactured from by hot compression molding out on a hydraulic press and a heated mold [5]. Three types of composite samples were manufactured:
PEEK/2D/CF - samples made of 2D carbon fiber cloths;
PEEK/2D/CF/CNT- samples made of 2D carbon fiber cloths modified with CNT;
PEEK/MD/CF made of chopped carbon fibers- reinforced PEEK;
The composite samples were obtained by hot molding of PEEK/CF prepregs. Mechanical durability of the samples was studied by aging them in Ringer's solution at 37°C and dynamically loaded under bending up to 10^6 cycles. The ultrasonic wave propagation method was applied to study changes in the composites. Biological tests were carried out in the presence of hFOB-1.19-line human osteoblasts and HS-5-line human fibroblasts. The level of collagen I produced by the cells was determined by ELISA test.

Results and Discussion

FIG. 1 shows changes in the dynamic elastic modulus of the CNT modified-composite plates and without CNT subjected to dynamic bending up to 10^6cycles.

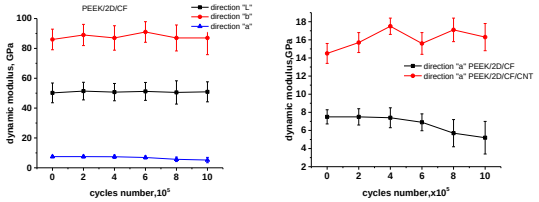

FIG. 1. Variations of dynamic elastic modulus of composite samples with and without CNT in function of cyclic bending loads; a denotes direction perpendicular to composite plate, b- along the width, L- along the length of the plate.

FIGs 2-5 show results of biological tests of composites with two types of carbon fibers and for the pure polymer. Cell viabilities were determined after 7 days cells culture.

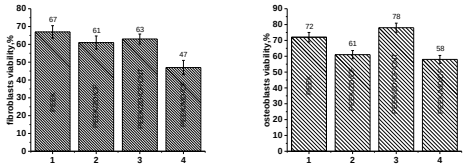

FIG. 3.Viability of fibroblasts and osteoblasts on composite surfaces on day 7 after seeding; TCPS as control.

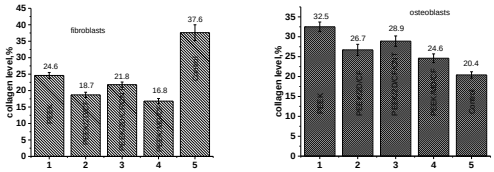

FIG. 4. Levels of collagen I produced by fibroblasts and osteoblasts on PEEK-based sample surfaces, normalized to the level of control (TCPS).

Conclusions

The changes in the mechanical stability of the composite samples were not significant after fatigue testing up to $1*10^6$ cycles. The tests showed differences between the samples in cells viability and levels of the produced collagen I.

Acknowledgments

The work has been supported by the National Science Centre project No. 2017/25/B/ST8/02602 and by the Polish National Centre for Research and Development, project no N N507 463437.

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RELAXATION BEHAVIOR OF ACRYLAMIDE-ALGINATE (AAM-ALG) WITH DIFFERENT MOLARITY OF CALCIUM CHLORIDE ($CaCl_2$)

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[ENGINEERING OF BIOMATERIALS 153 (2019) 63]

Introduction

Hydrogels are three-dimensional polymeric networks that include porous, hydrophilic, physical and chemical crosslinking [1-3]. Polyacrylamide (AAM) is one of the common materials that are used in variety of tissue engineering areas, but the pure AAM have extremely poor mechanical properties and biocompatibility [4–5]. Because of these shortcomings, the mechanical properties of AAM should be increased. One option is combining with other biomaterials such as alginate. Previous investigations have been indicated that the mechanical properties of pure AAM should be improved by synthesizing as double network with Alginate (ALG) [59–61]. We prepared AAM-ALG hydrogels containing various amount of crosslinker (BIS) and concentration of calcium chloride solution. Covalently crosslinked AAM and ionically crosslinked ALG contribute for extremely stretchable and tough properties on the hydrogels

The objective of this study is to conduct some experiments for determining of loading-unloading, creep, recovery and relaxation behavior through Polyacrylamide_Alginate (AAM-ALG) hydrogels that include different amount ionic and covalent crosslinker.

Materials and Methods

To understand the effect of crosslinking agents in the network we used different concentrations of $CaCl_2$ and N,N-methylenebisacrylamide (BIS). Hydrogels were synthesized by making an ALG and AAM solution with 1:8 weight ratio of alginate to acrylamide. The final concentration of ALG and AAM in the solution was 14% w. The ALG solution was heated in the oven at 121°C for one hour and cooled down at room temperature. It can be observed that the volume of the solution decreases after this step.

AAM and ALG solutions were mixed together. BIS of three different molar ratio 0,018 mol%, 0.028 mol% and 0,037mol% and tetramethylethylenediamine (TEMED) of 0.917 mol% relative to acrylamide monomer were added. After one hour the polymerization process is accomplished, the material is carefully removed from the mold and submerged into calcium chloride ($CaCl_2$) solution (0.05M, 0.1M and 0.3M) for alginate crosslinking. The uniaxial compression loading behavior of AAM-ALG was determined by using DMA Q800 (TA Instruments). Compression tests were carried out for different stress/strain levels.

Results and Discussion

A series of uniaxial compression loading tests were performed on three different AAM-ALG specimens that were prepared in 0.05, 0.1 and 0.3 M $CaCl_2$ solution at room temperature. The specimens are loaded up to 20% strain and hold at the same strain level for 5 minutes. Uniaxial stress-strain behaviors of AAM-ALG at room temperature on 0.05, 0.1 and 0.3 M $CaCl_2$ specimens are depicted in FIG. 1. The concentration of $CaCl_2$ solution dependency is clear; increasing molarity of $CaCl_2$ yields an increase in the stress level. Trends of these stress–strain curves are approximately the same. All specimens demonstrated hyperplastic behavior.

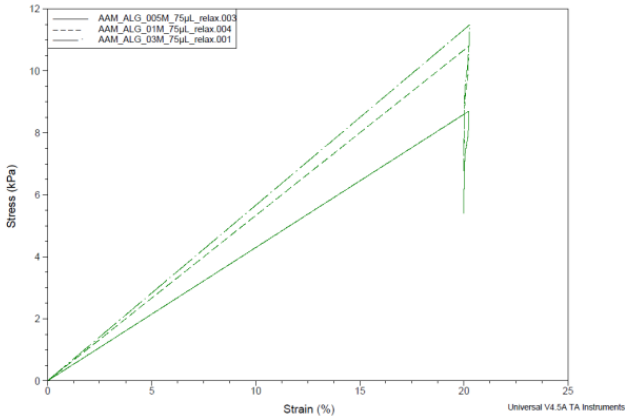

FIG. 1. Comparisons of loading behavior of AAM_ALG. Molarity of $CaCl_2$ solution are 0.05, 0.1, 0.3 M.

Relaxations at the strain levels of 20% were performed for 300 s to investigate the influences of concentration of $CaCl_2$ on the relaxation behavior. Stress versus time curves during relaxation tests at the strain levels of 20% are depicted in FIG. 2.

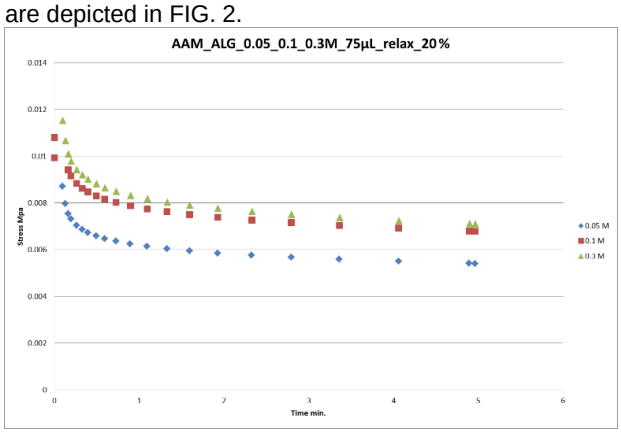

FIG. 2. Comparison of relaxation behavior of AAM-ALG at 20% strain level. Molarities of $CaCl_2$ solution are 0.05 M, 0.1, 0.3 M.

Conclusions

Observations are reported on AAM-ALG hydrogels in uniaxial compression loading tests with mixed program (loading a maximum strain 20% and hold for 300 s) at room temperatures. We used three different AAM-ALG that are 0.05, 0.1(75 µL BIS) and 0.3 M concentration of $CaCl_2$. Concentration of $CaCl_2$ solution dependency is clear; increasing molarity of $CaCl_2$ yields an increase in the stress level.

Acknowledgments

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GREEN - HYDROTHERMAL MICROWAVE SYNTHESIS AND CHARACTERIZATION OF HAP NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 64]

Introduction

Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is an inorganic component of bones and teeth. Hydroxyapatite possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues, probably due to its similarity with the hard tissues of the body. Nowadays hydroxyapatite is one of the most often applied bio-nanomaterials, e.g. in bone implants, scaffold layers, drug delivery agent, dental materials [1,2].

Materials and Methods

Hydroxyapatite nanopowder was synthesized by hydrothermal synthesis using microwave reactor MSS2 (Microwave Solvothermal Synthesis) [1,3]. The starting materials include pharmaceutical-grade substrates: calcium hydroxide $Ca(OH)_2$ and orthophosphoric acid H_3PO_4 as substrates to obtain ceramic nanoparticles. Nanopowder has been characterized by several methods: X-ray diffraction (Phase Purity), SEM (morphology), BET (Specific Surface Area) and helium pycnometry (Skeleton Density).

Results and Discussion

The unique, green process of microwave synthesis with strict control of the size of hydroxyapatite (GoHAP™) nanoparticles in the range of 10±1 to 42±4 nm have been shown. The control of synthesis parameters such as time, pressure and temperature allowed to control the particle size in a narrow distribution. The characteristics of GoHAP™ nanoparticles were compared with natural hydroxyapatite obtained from natural bones and tooth enamel. The high similarity of GoHAP™ nanoparticles to natural hydroxyapatite has been demonstrated.

Conclusions

Microwave synthesis allows easily and precisely control the grain size of nanoparticles. The size control of HAP nanoparticles gives the possibility of a better selection of the material properties for various applications. Particles of 40 nm show high similarity to HAP contained in dental enamel, so it can be successfully used in dentistry. GoHAP™ particles of 10 nm are almost identical to HAP particles contained in bones, therefore their use in bone tissue regeneration is proposed.

Acknowledgments

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ENZYMATIC, OXIDATIVE AND HYDROLYTIC DEGRADATION OF P(LA-b-TMC) COPOLYMERS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 65]

Introduction

Polylactide (PLA) is a well-known polymer that has been studied extensively for various biomedical applications due to its acceptable biocompatibility, inherent biodegradability, high modulus, and strength. Nevertheless, the high crystallinity of PLA results in poor elasticity and long resorption time of 2-3 years [1]. Poly(trimethylene carbonate) (PTMC) seems to be a perfect candidate to introduce elasticity to rigid PLA through blending or copolymerization; it is a rubbery and flexible material [2]. Copolymerization is a typical method of tailoring polymeric biomaterials properties because the specific architecture and composition of the copolymer can be easily obtained. Therefore, combined properties of a rigid chain from high Tg polyester with soft segments from rubbery polycarbonate can be achieved by introducing PTMC as a soft block into brittle PLA segments [3]. Such modification is also crucial in terms of biodegradation mechanism and kinetics of copolymers. In this work, we have focused on comparison of P(LA-b-TMC) copolymers with lactide (LA) contents of 70% and 50% upon degradation in three distinct environments.

Materials and Methods

P(LA-b-TMC) block copolymers with compositions LA/TMC 50/50 and 70/30 were subjected to enzymatic, oxidative and hydrolytic degradation. As controls, homopolymers of PLA and PTMC were used. Rectangular specimens of 50 mm × 5 mm were incubated at 37°C in pH 7.4 phosphate buffered saline (PBS) or in buffer containing 0.2 mg/ml lipase (refreshed every 3 days). Oxidative degradation was performed in 3% hydrogen peroxide solutions with 60 mM Co^{2+} at 37°C, refreshed once a week. Samples were periodically removed from the incubation solution, washed with deionized water, vacuum dried at room temperature and subsequently investigated by physicochemical methods. Tensile properties testing was complemented by the evaluation of mass loss, water uptake, contact angle, morphology (SEM), molecular weight of polymer (GPC) and thermal properties (DSC). Data are discussed in comparison with ^{1}H NMR results which allowed to follow changes of the composition and average sequence distribution of LA/TMC components.

Results and Discussion

Enzymatic degradation. Lipase is effective for degradation of PTMC-based homo- and copolymers, whereas lactide is resistant to this enzyme – no significant mass loss was detected in neat PLA after 17 weeks. In contrast, the copolymers exhibited various degradation rates. Mass loss of P(LA-b-TMC) 70/30 was slower than that of 50/50 which reached nearly 3% (17 weeks). The highest mass loss (18%) was observed for the PTMC homopolymer. The composition of the copolymers remained almost unchanged during the degradation period. On the other hand, the Mn of the P(LA-b-TMC) copolymers slightly decreased, while that of PTMC remained nearly constant during enzymatic degradation. Enzymatic degradation is a surface erosion process which does not affect the bulk properties yet, the hydrolysis still takes place in the bulk of copolymers.

Hydrolytic degradation. PTMC homopolymer degrades extremely slowly as no mass loss was detected. In contrast, P(LA-b-TMC) copolymers appear degradable through hydrolysis. No mass loss was observed for P(LA-b-TMC) 70/30 during the first 6 months, while the mass loss of P(LA-b-TMC) 50/50 decreased 3% after only 2 months. Hydrolytic cleavage, of, as presumed, ester linkages in copolymer chains, starts immediately after immersion in PBS, leading to rapid Mn decrease. Nevertheless, significant mass loss is observed much later, i.e., beyond 6 months. Mn of the copolymers significantly decreased, from c.a. 20 to 6 kDa, from 6 months to 12 months. The degradation products, i.e. low molecular weight species are thus formed and released into the medium, which resulted in rapid mass loss. LA units are preferentially degraded along the copolymer chains, leading to compositional changes.

Oxidative degradation. During the first 5 weeks all samples exhibited negligible weight loss < 0.5% and also small degradation rate based on the GPC results. Thereafter, the degradation rate becomes different for samples with various LA contents. For PLA and samples of LA/TMC molar ratio 70/30 a small weight loss below 1.0% is detected at 15 weeks. In the case of P(LA-b-TMC) 50/50 and PTMC, apparent weight loss is detected from 10 weeks, 2.0% and steadily increases to 5.0% at 15 weeks. In contrast to the weight loss, the molecular weight of PTMC sample and P(LA-b-TMC) 50/50 remained constant for 10 weeks; further a slight decrease in Mn was observed. In the case of PLA and P(LA-b-TMC) 70/30 a substantial drop in molecular weight was observed after 5 weeks, which was reflected also in the mechanical properties of the studied materials.

Upon degradation in all types of media, PLA and P(LA-b-TMC) 70/30 became brittle and eventually fell apart. PTMC and P(LA-b-TMC) 50/50 samples deformed – attained globular shape, became soft and adhered to the incubation vessel wall. In the course of degradation, the surfaces of those two samples became rough and a highly hollow structure was detected after 5 weeks, the size and depth of the hollows increased with the incubation time.

Conclusions

In this study, we have demonstrated that homopolymers and their copolymers degrade differently, depending on their morphological and chemical composition and the nature of incubation medium. Given this knowledge, we are able to tailor not only mechanical properties but also degradation characteristics by combining PLA backbones with TMC segments. The toolbox of techniques that has been used to study the degradation of biomaterials can be applied and employed to select biomaterials that are going to be used for pre-clinical in vivo studies with regard to a variety of clinical applications.

Acknowledgments

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BIOCOMPATIBILITY STUDY OF IMPLANTABLE DEVICE FOR THERAPEUTIC DELIVERY STERILIZED BY ETHYLENE OXIDE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 66]

Introduction

The purpose of the work was to select the most suitable sterilization method and evaluate biocompatibility by in vitro methods of the bio-electronic implant intended for therapeutics delivery secreted by confined genetically engineered cells upon stimulation by light (FIG. 1).

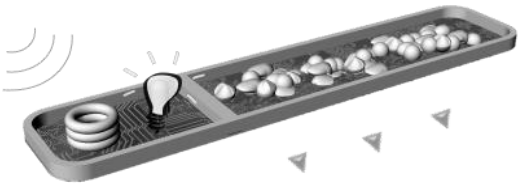

FIG. 1. A concept of wireless-powered cell-based implant for therapeutic delivery[1].

The device should fulfil essential requirements, such as mechanical and chemical stability in physiological environment during treatment of a disease or during their lifespan, and then be explanted or gradually degraded. In order to guarantee proper operation of such systems, especially in the case of their complex configuration or multifunctional tasks of the implant (e.g. active medical devices), besides selection of biomaterials and involved manufacturing processes, the designer should also consider potential sterilization method. Validation of selected sterilization technique in terms of its effectiveness, reliability and reproducibility is the prerequisite the manufacturer demonstrates to the notifying authorities in order to prove microbiological safety of the device.[2] Biocompatibility assessment is a part of validation of materials and components, including electronics towards following in vivo functionality testing.

Materials and Methods

Knowing the properties of polymeric materials comprising the implant, the complexity of its design and the presence of sensitive components and subsystems (FIG. 1), the ethylene oxide method was selected and applied for its sterilization. The device should be provided sterile for cells loading, therefore terminal sterilization of manufactured implant or aseptic assembling of pre-sterilized components may be applied. Validation of ethylene oxide sterilization method for the device under development was accomplished by overkill approach. In order to evaluate the biocompatibility of the components and the entire implant against human fibroblast cells (PCS-201-012™ ATCC) in vitro test recommended by the ISO 10993 – as for 'permanent implant contacting with tissue' – were carried out using XTT test, Live/Dead viability-cytotoxicity method, 2D-DIGE electrophoresis following changes in the proteome of the cells, and alternatively with irritation assay using skin model ex vivo.

Results and Discussion

A number of both physical and chemical processes can be used to properly sterilize a medical device, however steam sterilization, dry heat sterilization, chemical sterilization using gases like ethylene oxide, and radiation are among the most common. The choice of sterilization technique depends on the material composition of the medical device, how it's classified, and its intended use. Ethylene oxide (EO) is a chemical sterilization method usually suitable for complex or multi-material/component medical devices and was selected for current optogenetic implant. Because unlike steam sterilization and dry heat sterilization which require the medical device to be heat stable, a variety of materials – like plastics and electronic components – can be exposed to ethylene oxide without distorting the medical device form or its functioning. In the case of EO sterilization, besides rapid pressure changes, a dissolution of the gas (highly toxic) in the polymeric biomaterial and possible chemical reactions with the polymer should be examined.

Therefore, once the sterilization method that best suits a particular medical device is chosen, it is validated. Process Qualification at validation of EO method was demonstrated by application of sub-lethal, half and full sterilization cycles with the use of biological indicators confined in the cell chamber of the optogenetic implant. Moreover, the implant was examined to ensure that the applied sterilization procedure has not an adverse effect on the quality or integrity of the device or its components.

The biocompatibility of the materials after processing, and manufactured subcomponents comprising the implant, and the whole implant were examined by several in vitro assays. The cytotoxicity and viability showed no toxic effects towards fibroblast. The test to evaluate changes in cells proteome, comet assay and the potential to irritation gave results, as compared to controls, allowing qualification the device as biocompatible within the applied test conditions – no adverse effects were detected.

Conclusions

The applicability of ethylene oxide sterilization method for the developed implantable bio-electronic device for therapeutics delivery was presented. The biocompatibility tests results verified that the materials and components comprising this complex device, combined with the method of EO sterilization, are safe and can be applied in animal trials and in further clinical studies.

Acknowledgments

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CELLULAR INTERNALIZATION ANALYSIS OF FOLATE-TARGETED PLA-PEG FILOMICELLES LOADED WITH NEW BETULIN DERIVATIVE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 67]

Introduction

The use of natural plant-derived compounds has been considered to be an interesting aspect for the treatment of human neoplastic diseases because they are relatively easily available due to their commonly occurrence in the nature. Betulin has been shown to elicit anticancer properties by inhibiting cancer cells growth [1]. Moreover, series of new betulin derivatives have been synthesized, which characterize better cytotoxicity compared to betulin [2]. However, these agents are poorly soluble in water and it is important to develop dosage form that can effectively solubilize drug but also provide biocompatibility. Further progress is needed to develop bioresorbable carriers for targeted delivery of betulin derivatives which could be used for treatment of different kinds of tumors. PLA-PEG filomicelles were developed for targeted delivery of anticancer compounds. Folic acid (FA) was used as a targeting moiety. Thus, folate-drug delivery systems can enter cells by receptor-mediated endocytosis [3,4]. Investigation of uptake stage of micellar drug delivery system is crucial step to achieve effective anti-cancer therapy.

Materials and Methods

Filomicelles from the combination of poly(L-lactide)-Jeffamine-folic acid and poly(L-lactide)-poly(ethylene glycol) for delivery of betulin derivative 30-diethoxyphosphoryloxy-28-O-propynoylbetulin as an anticancer agent were prepared. HeLa human cervixadenocarcinoma cell line was used for uptake analysis. Flow cytometry and confocal laser scanning microscopy (CLSM) were used to study the cellular uptake of PLA-Jeff-FA/PLA3000PEG2000 micelles.

Results and Discussion

Fluorescence intensity of the HeLa cells incubated with PLA-Jeff-FA/PLA3000PEG2000 micelles with fluorescein was much higher than that of cells cultured in the presence of free fluorescein and the control with untreated cells.
CLSM analysis revealed subcellular distribution of PLA-JeffFA/PLA3000PEG2000 micelles with fluorescein in HeLa cells. Treated cells with folate-targeted filomicelles exhibited intense intracellular fluorescence. In contrast, almost no fluorescence was observed in the case of normal human connective tissue cells.

Conclusions

The successful internalization of PLA-Jeff-FA/PLAPEG micelles by FAR-positive HeLa cell line was confirmed by flow cytometry and confocal laser scanning microscopy. Effective cell uptake is a prerequisite to obtain carrier for efficient, targeted delivery of new anticancer agent and will allow to investigate internalization cell pathways.

Acknowledgments

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POLYSACCHARIDE-BASED HYDROGELS FOR BIOMEDICAL PURPOSES BY RADIATION-INDUCED SYNTHESIS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 68]

Introduction

Dextran is a non-toxic, bacterial polysaccharide mainly composed of linear α-1-6,linked D-glucopyranose residuals with a low content of α-1-2, α-1-3 and α-1-4-linked side chains [1]. Its biocompatibility and biodegradability are well documented, thus dextran has been extensively explored in the field of biomaterials [2]. Dextran has been clinically used for more than five decades as a plasma volume expander, it is able to decrease vascular thrombosis, reduce inflammatory response and promote vascularization, hence it is a perfect candidate for soft tissue regeneration. This biopolymer and nowadays its applications extend to new biomedical applications including hydrogel-like scaffolds for tissue engineering [2,3].

Chemical structure of dextran enables a wide range of chemical modification. Incorporation of crosslinkable moieties (e.g. methacrylic groups, -MA) into dextran structure results in derivatives capable for crosslinking initiated by UV or ionizing radiation [4,5].

The radiation technique is a very efficient and clean tool for modifying polymers. Unquestionable advantages of using radiation include possibility of processing materials in any physical state, at a convenient temperature (usually room temperature), typically with no need of application of additional chemicals, i.e. potentially toxic initiators or catalysts [6]. Moreover, if sufficient dose is applied (typically 25 kGy) the sterilization can be accomplished simultaneously with the processing. In our recent study we have demonstrated possibility of radiation synthesis of hydrogels based on biocompatible dextran methacrylate (Dex-MA) [6].

The current work was aimed to synthesize dextran-based hydrogels with addition of another biocompatible polysaccharide – sodium hyaluronic acid (NaHA), and to develop conditions suitable for formation of macroscopic polymeric network with use of radiation technique.

Materials and Methods

Dextran (from *Leuconostoc ssp*., Mr = 70,000) was purchased from Sigma-Aldrich (Canada), dimethyl sulfoxide (DMSO, 99.5%) and chloric acid (HCl, 36-38%) were obtained from Chempur (Poland). Glycidyl methacrylate (GMA 97%, stabilized by 0.005% hydrochinone monomethylether) was purchased from Sigma Aldrich, 4-(*N,N*-dimethylamino)pyridine (DMAP) were obtained from Sigma Aldrich (USA). Dextran derivatives have been synthesized using procedure of van Dijk-Wolthuis by coupling glycidyl methacrylate with this polysaccharide, yielding Dex-MA of various degrees of methacrylate substitution (DS) [7]. Synthesized Dex-MAs were characterized using FTIR and NMR spectroscopy. Dextran-based hydrogels were manufactured through polymerization/crosslinking of methacrylic groups of Dex-MA in aqueous solutions with radiation initiation. 3 and 5% aqueous solutions of Dex-MAs were prepared with addition of sodium hyaluronic acid (NuSci Pure Hyaluronic Acid HA Sodium Hyaluronate Powder, 91,6%) of two different molecular weights – 75 and 1029 kDa at concentrations of 0, 0.5 and 1%. Solutions of polysaccharides were deoxygenated and subsequently irradiated by electron beam (1 – 25 kGy). Following the irradiation, the samples of permanent chemical hydrogels underwent sol-gel analysis to determine basic parameters, i.e.: equilibrium degree of swelling in deionized water (EDS) and gel fraction (GF).

Results and Discussion

The main goal was to study the influence of NaHA additive on radiation-initiated synthesis of dextran-based hydrogels. Crosslinking of pure Dex-MA in aqueous solutions was found to be an efficient process yielding gels with high insoluble fraction content (up to 100 %). The swelling encompasses the wide range of 20 – 120 g of water absorbed per g of crosslinked polymer dependently on DS of used Dex-MA, and processing conditions. Addition of NaHA increase significantly swelling ability of the hydrogels, even up to 520 g of absorbed water. In general, hydrogels with NaHA are characterized by higher EDS, while retaining high content of insoluble fraction. Based on collected data it can be concluded that the utility characteristic of these polysaccharide-based hydrogels can be tailored by appropriate selection of parameters of polymer and the solution, such as molecular weight, DS, concentration, and irradiation conditions.

Conclusions

In this work, a series of Dex-MA was synthesized by the reaction of dextran with GMA. Irradiation of aqueous solution of Dex-MA with addition of sodium hyaluronic acid in absence of low-molecular-weight additives (crosslinkers) resulted in formation of macroscopic hydrogels even at doses as low as 100 Gy. Thus, obtaining hydrogel based on Dex-MA using ionizing radiation, i.e. crosslinking through unsaturated C=C bonds of -MA substituents, seems to be interesting alternative in comparison to other methods (chemical and UV-crosslinking). Moreover, it is possible to immobilize other biopolymers within crosslinked Dex-MA network. End-characteristics of these hydrogels can be tailored by manipulation of processing conditions. This, combined with well-known biological activity and functionality of dextran, and wide application of hyaluronic acid in the field of biomaterials implies possibility of biomedical applications of presented dextran-based hydrogel, especially in the field of soft tissue regenerative medicine.

Acknowledgments

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IMPACT OF ZrO_2 ADDITIVE ON PROPERTIES OF PMMA-BASED BONE CEMENT

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[ENGINEERING OF BIOMATERIALS 153 (2019) 69]

Introduction

Bone cements have been widely used in medicine for over 50 years mainly for fixation of the prostheses and for filling up small bone cavities in maxillofacial area or in spine vertebrae. There are two major types of bone cements commercially available – acrylic bone cements made out of poly(methyl methacrylate) and calcium phosphate cements (CPCs). PMMA-based cements are implemented because of their satisfactory mechanical properties, low toxicity, low cost and easy mass production [1]. CPCs are bioresorbable and biocompatible but they exhibit low mechanical strength [2].

PMMA-based bone cements are formed by mixing two components – solid part and liquid part. The basic solid component consists of poly(methyl methacrylate), benzoyl peroxide – an initiator and zirconium dioxide (ZrO_2) or barium sulphate ($BaSO_4$) – a radiopaque. The liquid phase involves methyl methacrylate (monomer), hydroquinone – a stabilizer, and N, N-dimethyl-p-toluidine – an accelerator. All mentioned ingredients are added to provide efficient polymerization at room temperature.

Zirconium dioxide (ZrO_2) can be considered for reinforcement phases in bioimplants because of its biocompatibility, exceptional oxidation resistance and wear resistance [3].

This paper presents the effect of zirconium dioxide (ZrO_2 = 6, 12, 21, 30 wt%) on physical, chemical and mechanical properties of novel PMMA-based bone cements.

Materials and Methods

Composite bone cement samples were prepared by mixing following components in solid phase – Duracryl (copolymer of methyl methacrylate and methyl acrylate), benzoyl peroxide, zirconium dioxide and the liquid phase – methyl methacrylate stabilized with hydroquinone and N, N-dimethyl-p-toluidine. The amount of ZrO_2 was variable in the sample types, from 6wt% to 30wt%.

The surface morphology of the bone cements was examined on JSM-6610LV Scanning Electron Microscope. For the studies of the chemical composition of the samples, Nicolet IS 50 FT-IR Spectrophotometer was used. Additionally, the wettability was measured via sessile drop method. Contact angles were measured with KRUSS Contact Angle Measuring Instrument and surface free energy (SFE) was determined according to the Owens-Wendt method [4]. Thermal properties were evaluated by means of two techniques – thermal gravimetric analysis (TGA, PI Instrument) and differential scanning calorimetry (Mettler Toledo DSC1). Moreover, mechanical properties were examined – Indentation hardness of the specimens was determined by means of durometer (Shore hardness scale D) according the norm ISO 868:2003, and Charpy impact tests were done.

Results and Discussion

The morphology of the samples showed that ZrO_2 tends to agglomerate. The higher content of ZrO2, the greater difficulty to obtain homogenous structure without defects.

In the FTIR spectra signals from matrix polymer bonds and groups present in ZrO_2 were recorded. The characteristic peaks for the Zr—O bond were found at 750cm^{-1}, 610cm^{-1} and 430cm^{-1}. Additionally, the peak at 460cm^{-1} and 700cm^{-1} exhibited higher intensity when ZrO_2 was added to the samples, which was the result of interaction of C-O-C bonds in the polymeric matrix.

Surface Free Energy was quite similar for all samples regardless of amount of ZrO_2 (SFE = 59.5±3.5 mJ/m^2; polar component = 16.2±2.7 mJ/m^2; dispersive component = 43.4±0.8 mJ/m^2).

The character of the degradation of the samples was evaluated via TGA analysis. The higher amount of ZrO_2, the higher temperature of initial and final degradation. For 6wt% of ZrO_2 10% degradation of initial sample mass was found at 272°C, for 30wt% – 294°C.

Polymerization heat was checked in the samples. For samples with 0, 6, 12 and 21% ZrO_2 no significant differences were found. With 30% ZrO_2 the polymerization heat decreased by 7J/g to 53J/g.

Addition of 21% and 30% ZrO_2 in the samples improved their hardness compared to the sample without ZrO_2. The smaller amount of additive worsened this property. ZrO_2 inclusion in PMMA-based bone cements had negative effect on their toughness, however, the correlation was not found. Further investigation is needed.

Conclusions

Addition of zirconium dioxide (ZrO_2) has an impact on final material properties. ZrO2 plays important role as radiopaque in bone cements but also it can improve implanted biomaterial. The higher amount of zirconium dioxide allows to improve the thermal behaviour of PMMA-based bone cements and to adjust mechanical properties in final cement.

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DOUBLE CROSSLINKING OF CHITOSAN/VANILLIN AS A BASIS TO MECHANICALLY STRONG GRADIENT HYDROGEL SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 70]

Introduction

Natural polymers have significant advantages over synthetic ones, e.g. biocompatibility, nontoxicity, and similarity to many biological structures what can be especially useful in tissue engineering [1]. Chitosan (CS) is one of the most abundant natural polysaccharides, its structure and properties are similar to those of glycosaminoglycans (GAGs) – natural components of the extracellular matrix (ECM). CS combined with an appropriate crosslinking agent can create chemically and mechanically stable hydrogels [2, 3]. Moreover, they can be easily modified to obtain materials with a range of various properties and hierarchical structures. This can be exploited in osteochondral tissue engineering, in which mainly the biochemical gradient has been reproduced so far, and new material solutions are still needed [4, 5]. This study aimed to evaluate the influence of double crosslinking of CS scaffolds with vanillin or tripolyphosphate on the hydrogel properties.

Materials and Methods

Chitosan (CS; Acros Organics, MW=100,000-300,000), and Avantor Performance Materials Poland S.A. reagents: acetic acid (AAc), vanillin (VAN), sodium tripolyphosphate (TPP) and ethanol (EtOH) were used as received. First, CS was dissolved in 2% AAc and vanillin was dissolved in ethanol were. Then, the VAN solutions were added dropwise to the CS and homogenised by sonication (10min). The final concentration of CS solution was controlled at 5% w/v. The mass ratios of CS:VAN were 1:0.8, 1:1, 1:1.2, 1:1.4, 1:1.6 and denoted as 0.8van, 1van, 1.2van, 1.4van, 1.6van, respectively. was mixed for 24h. The obtained hydrogels were maintained for 6 days at room temperature for effective crosslinking. After this time, 6x6 mm cubic samples of 1van, 1.2van, 1.4van were cut and immersed in vanillin solution (5% in ethanol, denoted as 1van_V, 1.2van_V, 1.4van_V) and TPP solution (5% in distilled water, denoted as 1van_T, 1.2van_T, 1.4van_T) for 24h to improve the crosslinking process. Some of the samples were frozen at -80°C and freeze-dried for further analysis.

Microstructural (digital microscope, SEM), structural (FTIR-ATR), mechanical (compression test), surface (wettability) and biological (biodegradability in PBS, bioactivity in SBF) properties of the obtained materials were evaluated.

Results and Discussion

Test results allowed to assess the influence of the amount and type of the crosslinking agent, the time of the crosslinking process and single vs double crosslinking process on the properties of the obtained scaffolds. Compression strength of the single (0.8van, 1van, 1.2van, 1.4van, 1.6van) and double (1.2van_V, 1.2van_T) crosslinked samples is shown in FIG. 1.

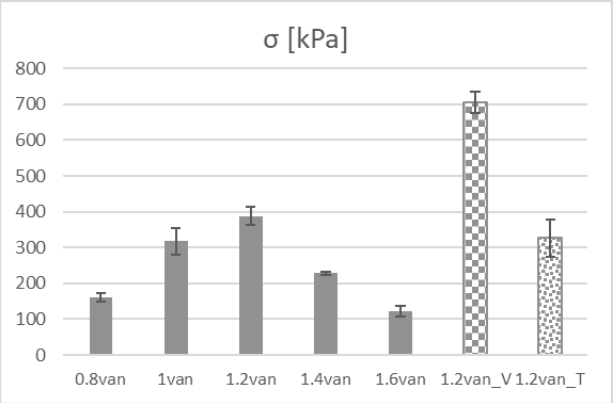

FIG. 1. Compression strength of the obtained hydrogel after single and double crosslinking.

For the single crosslinked samples, the highest compression strength was observed in the case of 1.2van. Double VAN-VAN crosslinking caused twofold increase of the compression strenght. Also, increase of the chemical stability was visible in the double-crosslinked, VAN-VAN samples, as well as the decrease of the swelling ratio. Double crosslinking with a TPP solution as a second crosslinker did not cause such differences.

Conclusions

The double-crosslinked CS hydrogels with higher mechanical properties and chemical stability were obtained in this study with the use of vanillin as a natural crosslinker. In the next step, they will be modified with additives, such as hydroxyapatite or graphene oxide to obtain hierarchical structures for cartilage tissue engineering.

Acknowledgments

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PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF TITANIUM DIOXIDE FILMS PREPARED BY TWO DIFFERENT PHYSICAL VAPOUR DEPOSITION TECHNIQUES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 71]

Introduction

Preserving hospital sterility constitutes one of the major challenges of today's health care institutions. Unfortunately, it still often happens that a patient gets infected in a hospital. To prevent that, hospital space should be protected with bactericidal coatings. Such protection, among others, can be successfully provided by thin TiO_2 films [1]. Titanium dioxide belongs to the most frequently investigated transition metal oxides. Thanks to its physicochemical properties, for many years it has been focusing attention of the scientific community [1,2]. Its most abundant functions comprise a white pigment, an optical filter material and a photocatalyst, not to speak of numerous biomedical applications.

As a result of its illumination with light of appropriate wavelength, active forms of oxygen are formed on titanium dioxide surface, which then acquires a strong bactericidal character against various strains of bacteria [2,3]. Additionally, such a coating responds to light excitation by changing its character from hydrophobic to strongly hydrophilic. That phenomenon is known as superhydrophilic affect and it, to a large extent, prevents TiO_2 surface contamination with solid particles [1-3].

There is a number of different synthetic routes leading to a deposition of titanium dioxide films described in the literature. The most frequently used techniques comprise sol-gel and anodizing methods as well as thermal spraying and reactive magnetron sputter deposition [3]. For a number of years, there has been an interesting novel modification of the latter technique developed in Poland [4]. This method, known as gas impulse magnetron sputtering (GIMS), consists in a repeated application of working gas pressure impulses of a magnitude enabling initiation of a glow discharge. The aim of the present work is to report a structure as well as water wetting and bactericidal properties of titanium dioxide coatings synthesized with both conventional reactive magnetron sputtering (RMS) and novel GIMS methods.

Materials and Methods

In both instances, the reactive sputtering of a titanium target in oxygen atmosphere was carried out. For the RMS processes, pressure and flow rate of oxygen were kept constant, thus allowing for the maintenance of a continuous discharge on magnetrons. In the case of the GIMS technique, on the other hand, oxygen was introduced in rapid pressure pulses which caused a pulsed discharge on the magnetrons. In both techniques the same rectangular planar magnetron and medium frequency power source were used. Both RMS and GIMS processes were carried out with an applied power of 0.8 kW and 1.5 kW.

Elemental composition and chemical structure of the films were investigated with the help of X-ray photoelectron spectroscopy (XPS), using a Kratos AXIS Ultra XPS spectrometer equipped with a monochromatic Al Kα X-ray source. In addition, chemical bonding of the films was studied with the use of Thermo Scientific model Nicolet iS50 Furrier transform infrared (FTIR) spectrometer. The phase composition of the coatings was investigated using a low angle grazing incidence X-ray diffraction (GIXRD) technique. For that purpose, a PANalytical Empyrean diffractometer with a goniometer diameter of 240 mm, working in the Bragg-Brentano geometry and utilizing filtered Cu KαX-ray radiation, was applied. Surface morphology of the coatings was studied with the help of scanning electron microscopy (SEM), using JEOL JSM-6610LV microscope. Water wetting angles were measured by means of Kruss, EasyDrop apparatus. Changes of water wettability under the effect of UV-B light illumination were followed in five minutes long steps. The bactericidal effect of the illuminated coatings was studied using *E. Coli* bacterial strain.

Results and Discussion

Elemental composition studies of the coatings show that they are predominantly built of titanium and oxygen atoms, with their Ti:O ratio being close to stoichiometric TiO_2. Apart from that, also oxygen bound carbon atoms are present at their surface, which constitutes a likely result of photocatalytic effect typical for that material. FTIR analysis of the films RMS deposited at the higher power level reveals the presence of a distinct sharp absorption band at 440 cm^{-1}. This band, corresponding to stretching vibrations of Ti-O bonds in anatase crystalline environment, in the case of GIMS synthesized samples appears already at the lower discharge power of 0.8 KW. As opposed to that, an application of the lower power level in the RMS process results in a broad, weakly separated band present in that region and revealing an amorphous character of the film. All the above results of the coatings phase composition were confirmed with the XRD data. SEM studies have shown a smooth and homogeneous surface of the films. Water wettability measurements under the influence of UV-B light revealed a strong hydrophilic effect in the case of RMS samples deposited at 1.5 kW of discharge power as well as in that of GIMS synthesized films deposited at 0.8 kW. In all cases, microbiological studies showed a substantial bactericidal effect, ranging from 21% to 50%.

Conclusions

The results acquired in the present work show that high quality homogeneous TiO_2 coatings can be produced with both RMS and GIMS methods. All the samples exhibit photocatalytic as well as photowetting effect. In the case of films synthesized with the GIMS technique, the strongest effect is observed for samples deposited at 0.8 kW of magnetron discharge power, while a double of that magnitude is needed to attain a similar level of photoactivity of the coatings produced with the help of RMS method.

Acknowledgments

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DIFFERENT BEHAVIOUR OF ADIPOSE TISSUE-DERIVED STEM CELLS AND VASCULAR SMOOTH MUSCLE CELLS ON MODIFIED POLY(L-LACTIC ACID) FOILS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 72]

Introduction

Nowadays, damaged blood vessels can be replaced by biological grafts or synthetic prostheses. Due to the limited availability of biological grafts, current vascular tissue engineering focuses on various modified polymers with mechanical and biological properties similar to blood vessels. Poly(L-lactic acid) (PLLA) is a biocompatible and slowly degradable polyester; however its relatively high hydrophobicity can impair cell-material interactions as well as later positive interactions with surrounding tissues in human body [1].

Vascular smooth muscle cells (VSMCs) are a major component of the *tunica media* (i.e. a middle layer of blood vessels). Adipose tissue-derived stem cells (ADSCs) are mesenchymal stem cells which can be harvested in large quantities and can be later differentiated towards VSMCs by medium composition, biomaterial properties and/or dynamic culture conditions [2].

The aim of our work was to study the growth, behaviour and differentiation of ADSCs and VSMCs on variably modified PLLA foils which can be later used for vascular tissue engineering purposes.

Materials and Methods

Biopolymer PLLA (crystallinity of 60-70%, density of 1.25 $g.cm^{-3}$, the thickness of 50 μm (± 20 %), purchased from Goodfellow, UK) was used. The PLLA were variably modified and the studied samples were as follows: pure PLLA, plasma-treated PLLA (PLLA240), plasma-treated PLLA grafted with polyethylene glycol (PEG), plasma-treated PLLA grafted with dextran (Dex), and control tissue culture polystyrene (PS).

The samples were sterilized in 70% ethanol for 1 hour, washed with PBS, and then the ADSCs or VSMCs were seeded in a growth medium, i.e. DMEM + 10% foetal bovine serum (FBS). The growth medium was partly changed with differentiation medium on day 4 (for ADSCs) or on day 7 (for VSMCs). The differentiation medium contained DMEM + 2% FBS, TGF-ß1, and BMP4, and was changed twice a week with an overall culture time of 3 weeks.

The metabolic activity of the cells was measured by resazurin conversion assay. The immunofluorescence staining was used to visualise initial cell morphology and to detect the early, mid-term and late markers of differentiation towards VSMCs, i.e. α-actin, calponin and SM-myosin heavy chain (SM-MHC). The differentiation was quantified by RT-qPCR.

Results and Discussion

The initial metabolic activity of ADSCs was similar on all samples. Moreover, this tendency was the same in later time intervals when the cells were cultured in the growth medium. However, when the differentiation medium was added to ADSCs, the metabolic activity was higher on PLLA240 and control PS than on pure PLLA, PEG and Dex samples. The initial metabolic activity of VSMCs was the lowest on pure PLLA. The PLLA240 and control PS samples showed higher metabolic activity of VSMCs than the other samples. The addition of differentiation medium to VMSCs decreased the metabolic activity; however the trend of metabolic activity values remained the same. On day 1, the cell morphology revealed almost the same morphology characteristics of ADSCs on all samples (FIG. 1). In contrast, the VSMCs were visibly round and showed higher circularity on pure PLLA and Dex samples than on PLLA240, PEG, and control PS (FIG. 1).

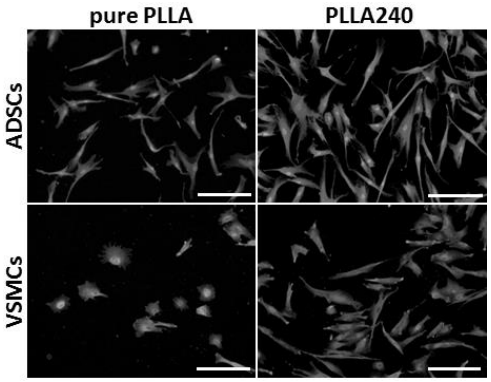

FIG. 1. The morphology of ADSCs and VSMCs on pure PLLA and plasma treated PLLA (PLLA240) on day 1 after seeding, scale bar 100 μm.

The addition of differentiation medium to ADSCs supported the differentiation towards VSMCs on all samples which was proved by the positive immunofluorescence staining of α-actin and calponin, and sporadically also by positive staining of SM-MHC. These results were quantified by increased gene expression of α-actin, calponin and smoothelin.

Conclusions

In our study, we proved the biocompatibility of pure and modified PLLA in three-week cell culture. The modification of pure PLLA improved the adhesion, metabolic activity, and growth of the cells. With appropriate medium composition, the differentiation of ADSCs towards VSMCs was successful on all samples. We observed different behaviour of ADSCs than of VSMCs on samples and also different behaviour within one cell type depending on the medium composition.

Acknowledgments

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COMPARISON OF SILVER AND COPPER AS DOPANTS OF CARBON COATINGS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 73]

Introduction

Scientists and doctors from around the world are constantly trying to find solutions to emerging problems associated with the use of implants, despite the noticeable process of improving currently used materials and introducing new ones. Still problematic are biological effects such as: release of metal ions from the implant or wear products into surrounding tissues, cytotoxicity and genotoxicity, but also formation of biofilm layers which may lead to severe inflammations.

Since initial and long-term behaviour to the implanted material depends largely on its surface interactions with the body, high impact is placed on approaches involving various types of coatings such as structures of TiO_2 [1] or diamond like carbon (DLC) coatings [2]. Further improvement of desired features can be achieved by doping of such films. There are at least a dozen different admixtures of only DLC coatings reported by literature [3,4]. Among the elements of high bactericidal potential but still unsure effect on mammalian cells are copper and silver. The purpose of the following researches was to compare and contrast the effect of Cu and Ag as dopants of DLC coatings in potential biomedical applications such as coatings for intramedullary nails.

Materials and Methods

AISI 316 LVM was selected as a substrate material for all the coatings taking into consideration its wide use as a material for implants. Both Cu-DLC and Ag-DLC coatings were deposited by means of PVD process in which carbon matrix was originated from graphite source while dopant was introduced via sputtering of metal target.

The chemical composition of examined coatings was verified by means of X-ray photoelectron spectroscopy (XPS) examination conducted with AXIS Ultra DLD (Kratos Analytical) system and Raman spectroscopy performed with help of InVia (Renishaw) apparatus. Mechanical properties were evaluated using Nano Indenter G200 (Agilent Technologies, USA).

During evaluation of the bactericidal effect of Ag and Cu, the liquid culture of model organism *Escherichia coli* was used. The number of bacteria adhered to the examined surfaces was determined by means of fluorescent spectroscopy of specimens treated with propidium iodide and bis-benzamidine.

Results and Discussion

Initial trials of radio frequency magnetron sputtering of Cu and Ag targets with powers from 5 to 25 W were associated with a significant reduction of the hardness of the deposited coatings in relation to undoped DLC. In most cases, the hardness of coatings was on a similar or lower level than the one observed for substrate material. Also the results presented by other research groups showed depletion of hardness especially with addition of silver [5]. Probably those coatings were in fact composites of metal with only a small addition of carbon, similar to the coatings produced by Khamseh et al. [6]. The satisfactory hardness (resembling value of DLC) was achieved by introducing screens that limit the effective sputtering surface of the dopants' targets. Finally the coatings with amount of Cu of up to 7 at.% and Ag of up to 10 at.% were synthetized. Some reduction of hardness with high amounts of dopants was related with increasing fraction of bonds with sp2 hybridization.

The biological examination revealed a reduction of the number of adhered bacteria on both Ag-DLC and Cu-DLC but only at certain level of dopant. In literature, a significant reduction of biofilm formation was achieved at amounts of copper of at least 9 at.% [7], which is higher than maximum amount of that dopant examined in the following work. The bactericidal potential of coatings with silver was higher than in case of addition of Cu to the carbon matrix, which is in agreement with the results of Goudouri et al. [8] conducted for metals.

Conclusions

Conducted researches prove the possibility to deposit Cu-DLC and Ag-DLC coatings with hardness not lower than for substrate material and for low amounts of dopant resembling even the value for undoped coating. Both tested dopants elements can reduce biofilm formation, but the bactericidal effect is dependent on the type of dopant and its concentration.

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- MEDGAL Sp. z o.o, 16-001 KSIĘŻYNO, ul. Niewodnicka 26A.

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BIOACTIVITY SAMPLES WITH SiO_2-Y_2O_3 CERAMIC LAYERS PRODUCED BY SOL-GEL METHOD

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[ENGINEERING OF BIOMATERIALS 153 (2019) 74]

Introduction
316L stainless steel is widely used in implantology, although biological complications may result from its insufficient mechanical properties and low corrosion resistance in the human body. In order to improve the corrosion resistance of 316L steel and its bioactivity, coatings are applied, e.g. ceramic layers that can improve the proliferation of living cells and barrier protection in the human body [1,2].

Materials and Methods
14.8 mm diameter samples of 316L stainless steel were used as a substrate. The application of the layers was based on the sol-gel technique. The coating solutions were obtained from tetraethoxysilane (TEOS) and yttrium(III) nitrate. As the solvent, butanol was used. Acetic acid and nitric acid were used to accelerate the reaction. The coatings were deposited using the immersion method. They were deposited in various sequences on 316L steel.
The thickness of the oxide layers was measured using SEM/Ga-FIB scanning microscope. The surface topography and shape were analyzed by SEM and also the same for distribution and morphology of MG-63 cells in the surface after 24 and 96 hours. In additionally, the proliferation analysis was carried out by using cell counting every 24 hours. However, the cytotoxicity effect was analyzed by using MTT assay every 24 hours to determine the toxicity effect of coatings to MG-63 adherent cells.

Results and Discussion
The photos showed correct morphology for MG-63 cells and their distribution was regular. The MTT test showed differences in cell proliferation on individual samples. Samples are not cytotoxic and have a good effect on cell proliferation. Electrochemical tests performed in SBF solution proved that the corrosion resistance of samples coated with SiO_2 and Y_2O_3 increased compared to uncoated 316L steel.

Conclusions
The produced sol-gel coatings of SiO_2-Y_2O_3 on 316L steel increase the barrier properties and bioactivity.

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HYDRO- AND SOLVOTHERMAL NANOPOWDER SYNTHESIS IN MICROWAVE REACTORS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 75]

Introduction
Nowadays more and more bioactive materials are used in medical applications, which can support osseointegration. Bioactive hydroxyapatite (HAP) is one of the inorganic component of hard tissues, which is manufactured in The Institute of High Pressure Physics (IHPP PAS) and it is called GoHAP™

Materials and Methods
Microwave solvothermal synthesis (MSS) is an example of microwave assisted wet chemical synthesis process and nowadays it is counted as one of the most popular chemical methods of obtaining nanomaterials, like HAP, ZnO, ZrO_2 and other. The morphology, grain size and specific surface area of the nanopowder can be controlled thanks to the microwave reactor and the high pressure consolidation technology for ceramic materials. Microwave heating enables better control of the reaction time, fast heating and reducing the thermal gradients. This results in a better crystallinity of the nanoparticles comparing to the precipitation process. An additional advantage is a reduced synthesis temperature, so no powder calcination is needed.

Results and Discussion
Nanohydroxyapatite was synthesized by precipitation method in room temperature and after heated by hydrothermal synthesis using microwave reactor MSS2. In the IHPP PAS we are able to synthesize and obtain six types of hydroxyapatite with different crystallinity degree and grain size [1].

Conclusions
At the Laboratory of Nanostructures, IHPP PAS, we have been developing new type microwave reactors for nanopowders synthesis for more than 15 years. The use of the microwave radiation and the unique design of the reactors permit precise pressure control during the quick synthesis processes, controlled with the accuracy of even one second. The reactors like MSS1 or MSS2 also present a control system which allows for automatic operation in the stop flow mode or use the batch (closed vessel) mode in bigger production scale than in other commercial equipment [2]. In the MSS2 reactor in the stop flow mode, the process chamber is emptied exactly upon finishing the heating, which results in a rapid cooldown and freezing of the reaction. The batch system brings inertial purity and repeatability of the process of medical purity nanopowders. And also the same concentration of the each batch of synthesized product [3].

Acknowledgments
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CYTOCOMPATIBILITY TESTS OF POLY(DIOL CITRATE) POLYMERS IN CONTACT WITH ADIPOSE TISSUE DERIVED STEM CELLS FOR VASCULAR TISSUE ENGINEERING PURPOSES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 76]

Introduction

Poly(diol citrates) (PDCs) represent a new generation of advanced biocompatible and biodegradable synthetic materials with potential application in vascular tissue engineering [1]. PDCs synthesis is realised by polycondensation and they have tunable mechanical properties and degradation rate [2]. The aim of our study was to synthesise PDCs of various diols and molar ratios and to characterize their cytocompatibility. Due to the large number of functional pendent carboxyl and hydroxyl groups, these materials can be further modified to provide them with other properties, e.g. fluorescent or anti-oxidant, which are of importance in biomaterials engineering.

Materials and Methods

Citric acid was reacted with various diols in molar ratio of 1:1, 2:3 and 1:2, creating PDCs. Reagents were added to 30-ml flask and melted at 140°C for 40 min under stirring to synthesize prepolymers. Then, the prepolymers were dissolved in ethanol, precipitated in water, lyophilized and post-polymerized for 10 days at 80ºC. Different length of diol chains were used in the synthesis, i.e. hexanediol (PHC), octanediol (POC), decanediol (PDC), and dodecanediol (PDDC). To observe potential cytotoxicity of these materials, we prepared 10% extracts in cell culture medium (DMEM, 24 h, 37°C). The pH of the extracts was measured prior to their addition to culture wells. Meanwhile, human adipose tissue-derived stem cells (ASCs, isolated from lipoaspirates under ethical approval issued by the Ethics Committee in the Hospital Na Bulovce in Prague, and under informed consent obtained from the patients) were seeded at a density of 4,500 cells/well into 96 multiwell plates. The extracts from the materials were sterilised by 0.22 microns filter. Next day after cell seeding, the undiluted (1:1) and diluted extracts at a ratio 1:2, 1:4, 1:8, and 1:16 were added to wells with ASCs. The cytotoxic influence of the extracts was measured by Alamar Blue assay and the cells were visualised with live/dead staining; both procedures being made on days 1, 3 and 7 after the extract addition.

Results and Discussion

The pH measurements of extracts revealed decreased values in all samples as compared to the control medium. The values were the lowest in the case of hexanediol-based samples (pH from 5.31 to 6.50), and they generally increased with the longer chain of carbohydrates (pH up to 7.36). The molar ratio of diol to citric acid also influenced the values – the higher content of citric acid, the lower the pH was. The live/dead staining revealed almost all dead cells in the undiluted extracts (i.e. 1:1 dilution) except for PHC_2:3, PDDC_2:3 and control medium where the cells were alive. In the dilution of 1:2 to 1:16, the cells were alive in almost all tested samples.

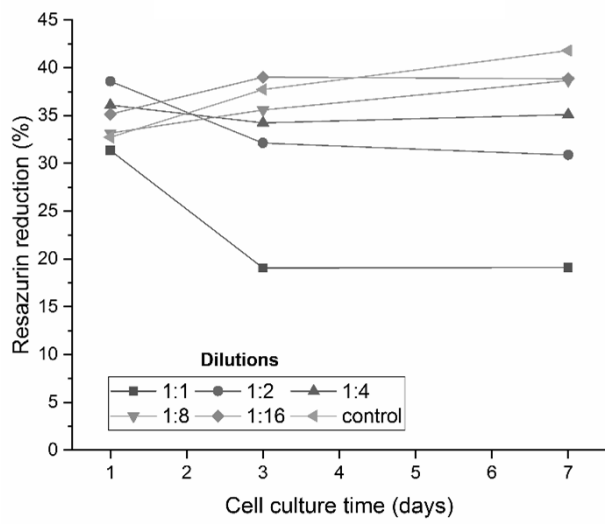

FIG. 1. Resazurin reduction (Alamar Blue test) of ASCs cultured for 1, 3 and 7 days with various dilutions of extracts of PHC_2:3 in DMEM.

The results from Alamar Blue assay confirmed those obtained from live/dead staining. Undiluted extracts (1:1) were the most cytotoxic with the resazurin reduction level at 0% (except for PHC_2:3, PDDC_2:3, PDDC_1:2). In dilution of 1:2 although the cells were alive, their viability decreased during 7 days. The more diluted the extract, the higher cell viability was. In dilutions from 1:4 to 1:16, the viability of cells was almost on the level of control samples. Among the studied samples we observed the lowest viability on decanediol-based samples. The least cytocompatible material was PDC_1:2. The highest viability of cells was found for PHC_2:3 (FIG 1.) and PDDC_2:3.

Conclusions

Various polymers differing in the ratio of citric acid to diol were synthesised and their cytocompatibility was characterised. The specific diols and specific molar ratio of diol to citric acid influenced the pH values of culture medium and viability of cells. However, it seems that there are potentially other determinants which can influence the viability of the cells except for pH values. Based on the results, we chose PHC_2:3 for direct cell seeding onto polymer.

Acknowledgments

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POLYURETHANE-BASED HYDROGELS MODIFIED WITH GRAPHENE OXIDE AND HYDROXYAPATITE FOR CARTILAGE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 153 (2019) 77]

Introduction

The properties of polyurethane-based porous hydrogels can be widely modified by selection of substrates and other additives to fulfil requirements of cartilage tissue engineering. The proper choice of the building blocks like polyols, isocyanates, chain extenders/crosslinkers can be implemented to obtain systems, which can mimic the native tissue. Moreover, they can be biocompatible and biodegradable. It has been described in the literature that biodegradable polyurethane (PU) porous scaffolds seeded with articular chondrocytes support cell attachment and the production of extracellular matrix proteins [1,2]. In our studies polyurethanes crosslinked with chitosan and modified by chemically grafted hydroxyapatite (HAp) were obtained in a one-step bulk polymerization.

Materials and Methods

Synthesis of PU with 50% of soft segments was carried out using 4,4'-diphenylmethane diisocyanate (MDI), and mixture of poly(ethylene glycol) 2000 (PEG) and poly(ε-caprolactone) (PCL) with 1:3 molar ratio as polyols, according to the procedure described in Ref. [3]. PU was obtained without catalyst. Various molar ratios of chitosan and 1,4-butanediol (BDO) were applied as crosslinking systems. PU systems were modified with graphene oxide (GO) and hydroxyapatite (HAp).
PU has been investigated using FTIR, DSC and TG methods. Moreover, microscopic observation, investigations of in vitro chemical stability, bioactivity and mechanical properties have been performed.

Results and Discussion

PU structure has been confirmed using FTIR method. In the FTIR spectra the absorption bands associated with –NH group at 3300 cm^{-1} in urethane group have been found. The double peak at ca. 1720 and 1680 cm^{-1} can be attributed to stretching vibrations of C=O in urethane groups engaged in hydrogen bond formation and free carbonyl groups. These results suggest phase separation in the PU matrix. The absorption band at 2250 cm^{-1}, due to the stretching vibration of -NCO groups, were not observed, which confirms the complete reaction of diisocyanates at the polymerization stage. The absorption bands in the range of 1040–1098 cm^{-1} can be attributed to the vibration of the PO_4^{3-} group. Moreover, no absorption bands from -OH groups in HAp can be found that suggests chemical bonding of HAp to PU matrix as well as no absorption bands from hydroxyl groups in the chitosan structure have been detected that confirm chemical bonding between chitosan and PU. As a result porous PU systems were obtained as it was presented in FIG. 1.

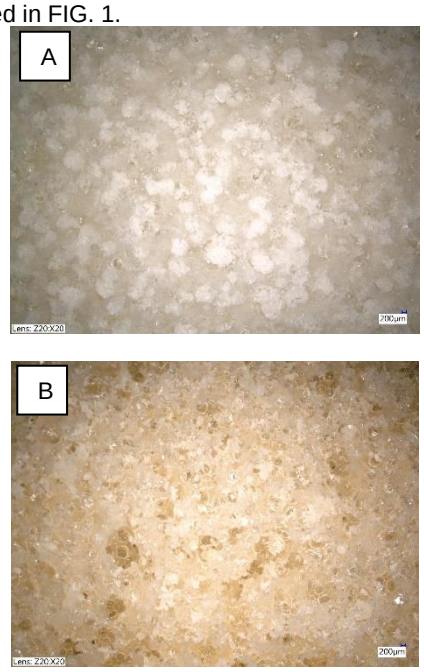

FIG. 1. Microphotographs of PU systems modified with 1 (A) and 5% (B) Hap.

Results of TG analysis are presented in FIG. 2.

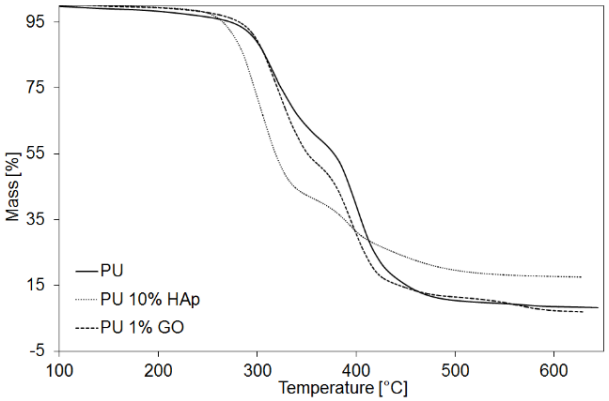

FIG. 2. TG curves of the obtained PU systems.

Conclusions

PU structure and successful polymerization of the isocyanate groups with hydroxyl moieties from chitosan and HAp were confirmed by FTIR methods while good dispersion and porous microstructure have been found using microscopic methods. Thermogravimetric results show that the presence of HAp and GO show a significant impact on the thermal properties of PU systems. It can be seen that HAp incorporation leads to decreasing in the thermal stability of PU systems compared to unmodified PU, while incorporation of GO changed degradation mechanism – the third degradation step has been detected for sample modified with GO.

Acknowledgements

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BIOMIMETIC TRIPHASIC CONSTRUCT FOR OSTEOCHONDRAL TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 78]

Introduction

According to the last United Nation report, over 130 million people will suffer from Osteoarthritis (OA) worldwide by 2050, of whom 40 million will be severely disabled by the disease. OA is a long-term chronic disease that often develops in patients affected by non-treated traumatic osteochondral lesions [1].
The lack of effective treatments for osteochondral tissue (OTE) repair can be ascribed partially to the structural complexity of this region: this, in fact, is characterized by a multilayered architecture comprising of non-calcified – also referred to as hyaline – cartilage, calcified cartilage, and subchondral bone. In the last decade, various scaffold-based approaches for osteochondral repair have been investigated. Osteochondral tissue engineering strategies are generally categorized into monophasic, biphasic and triphasic models according to the number of biomaterials or cells present in the engineered structures [2–4].
From an engineering point of view, it is very challenging to fabricate structure with simultaneously concerted biological and mechanical characteristics between soft, viscoelastic hyaline cartilage and hard, stiff subchondral bone. The aim of the study was to combine the advances deposition systems based on (i) 3D Bioprinting combined with sprayed cross-linking system, (ii) innovative deposition system based on coaxial-needle extruder developed in-house, (iii) fused deposition modelling supplemented with post-printed treatment in order to fabricate the triphasic construct that could tailor structure and properties of native osteochondral tissue.

Materials and Methods

Triphasic 3D construct is made of two bioinks (i) alginate (Alg) combined with short polylactide (PLA) fibers, (ii) alginate combined with gelatin methacrylate (GelMA) and ß-tricalcium phosphate particles that recapitulate cartilaginous parts of OTE. Corresponding to the subchondral bone we formulate the 3D scaffold with a potential to a stronger commitment toward early osteogenic differentiation of hMSC consisted of the 3D printed polycaprolacton scaffold subsequently modified with an innovative solvent treatment method based on acetone and ultrasounds impact.

Results and Discussion

The multilayered TC structure was successfully fabricated by advanced fabrication techniques. Distinct zones were subsequently assembled into TC to recapitulate the osteochondral tissue.
Our results demonstrate the possibility of joining individually fabricated tissues into one integral triphasic model characterized by the proper biological and mechanical response. We successfully bioprinted the chondrocytes and the mesenchymal stem cells encapsulated in the hydrogel parts mimicking native non-calcified and calcified cartilage, respectively. Moreover, obtained surface topography increased osteogenic potential of the subchondral specific scaffold zone, making the proposed approach the best candidate to be bone substitute. Moreover, the conducted structure examination and mechanical testing of the constructs confirmed their potential use in osteochondral tissue engineering.

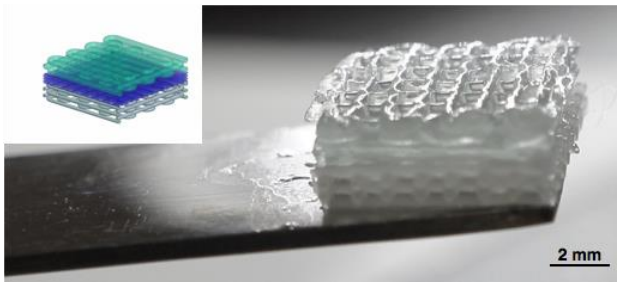

FIG. 1. Triphasic scaffold for osteochondral tissue engineering regeneration.

Conclusions

The proposed triphasic construct provides an integral load-bearing structure and provides the individual functionality edifying the zonal structure of native osteochondral tissues.

Acknowledgments

This study was financially supported by NCBR within the project Pol-Nor/202132/68/2013 and grant “subwencja” from the Ministry of National Education. We thank Xanofi Inc. for the supply of fibers.

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PROTEINS AND PEPTIDES OF INTERCELLULAR COMMUNICATION, STABILIZED WITH HYALURONIC ACID FOR TOPICAL USE TO RELIEVE INFLAMMATORY SKIN DISEASES AND PROMOTE WOUND HEALING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 79]

Introduction

Hyaluronic acid (HA) is a widely known glycosaminoglycan of large size, involved in many physiological processes in tissues. There is evidence that HA plays a role in various pathological pathways such as inflammation and cancer. CD44 is the main receptor for HA membrane cells.

We know that high molecular weight HA has anti-angiogenic, anti-inflammatory and immunosuppressive effects, while smaller HA oligomers are pro-angiogenic, pro-inflammatory and immunostimulatory. HA is also strongly involved in leukocyte recruitment in the area of inflammation [1].

On the other hand, proteins and peptides of intercellular communication (PP) like for example growth factors stimulate or suppress cell and tissue growth in the suppression process, affect gene expression, DNA replication or T cell production, significantly accelerating healing or affecting inflammation of skin tissues.

Modulatory factors are capable of modulating the immune response and thus help play a role in modulating the inflammatory process. Transferrins, an antiviral, anti-bacterial, iron-binding proteins, modulates cytokine release. Its receptors have been found on several blood and skin cells [2-4]. Cytokines such as interleukins (IL) regulate the duration and intensity of the immune response, boost T-cell activity.

Materials and Methods

In vitro: Cell proliferation studies were performed using HA stabilized PP and an immortalized epithelial cell line. To study the inflammatory effects of components containing unstimulated HA peripheral blood mononuclear cells (PBMCs) and stimulated with PBMCs by mitogen, they were incubated with the active ingredients of the prepared PP and HA mixture. The measurement of neopterin production and tryptophan degradation was carried out by HPLC. Because PBMCs are mainly composed of T cells and macrophages, measurements were made to gain insight into the interaction between T cells and macrophages. Viable cell measurement was performed using the Trypan Blue method.

In vivo: Patients were asked to apply a PP and HA mixture at least twice a day in the morning and at bedtime. The aim was to assess the efficacy and safety of the preparation in adults suffering from a number of skin conditions. 2 weeks before the study and during the study, patients did not use any topical preparations, steroids or phototherapy. The application was carried out on 120 patients.

Results and Discussion

The PP-HA gel has been shown to be effective for proliferation of an epithelial cell line, an important process in healing of damaged skin. The mixture has also been shown to be effective for a wide range of skin ailments. It heals difficult to heal post-operative wounds.

Healing of wounds may be due to the presence of several growth factors, identified in PP components, responsible for proliferation of normal epithelial cells. HA displays important biological properties and plays a significant role in crucial physiological processes especially when cellular plasticity is involved such as inflammation, immune reactions, angiogenesis, and wound healing. These varied physiological functions are related either to interactions with specific or less specific cellular membrane receptors or to the production of HA fragments of different sizes, generated for example by local traumatism or inflammation, fragments displaying specific properties according to their size [6].

HA was also shown to reduce TNF-a and morphological inflammation both in human A431 epidermoid skin cells and in mouse fibroblasts [6]. Studies have shown that hyaluronic acid [6][7] and PP interfere with immunopathogenic pathways which involve proinflammatory cytokines such as IFN- γ and TNF-a.

The suppressive influence of neopterin and knyurenine production by colostrum components and the suppressive effect of TNF-a by HA could explain the effects we have seen on patients with inflammatory diseases.

PP together with HA stimulates neopterin production and tryptophan degradation in unstimulated PBMC. This could be a way of treating some inflammatory diseases. PP and HA counteracts activation cascades in mitogen-stimulated PBMC (data not shown). This could be a way of treating other skin problems like psoriasis.

Acknowledgments

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EFFICIENCY IMPROVEMENT OF DRUG CARRIER DELIVERY NAVIGATED BY ENDOVASCULAR ADDRESSING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 80]

Introduction

Currently, in clinical practice, drugs that have a large number of side effects are often used. Such as cytostatic or immunomodulatory drugs lead to significant damage to vital organs and intoxication of the whole body. In order to reduce the drug side effects, various approaches have been proposed for the elaborate new types of drug delivery systems. It is necessary to develop a method for targeted drug delivery that can increase the therapeutic effect due to the prolonged release of the drugs without systemic side effects in the vital organs.

We have proposed a combination of modern endovascular surgery methods and targeted delivery of micron-sized polyelectrolyte microcapsules that sensitive to an external magnetic field in vivo. A region of interest was selected in which the drugs are delivered in a low concentration compared to the dose systemically administered. The magnetic-inducted microcapsules` targeting by using injection into the hindpaw femoral artery is significantly more effective than the tail vein injection. As also the use of micro-sized polyelectrolyte capsules that were delivered intra-arterially to the kidney, allows to increase the released drug concentration in the interest region in comparison with the intravenous administration way.

Results and Discussion

We described to using composite microcapsules for research a distant control in vivo by magnetic field gradient. The visualization of microcapsules in vivo was on stage by Near-Infrared Fluorescence Imaging for Real-Time. The microcapsules contain magnetite nanoparticles and fluorescent dye – Cyanine 7 NSH-ester conjugating with bovine serum albumin and polyarginine. The average size of the microcapsule was about 5±1μm. The microcapsule suspension injections into the tail vein (systemic administration) and into the femoral artery (local administration) was proposed out respectively. The magnetic targeting of microcapsules injected through the femoral artery was more efficient compared with tail vein injection. A small dose of micron-sized polyelectrolyte micron capsules was delivered to the kidney by introducing a capsule suspension through a catheter into the renal artery. Capsules have been shown to successfully linger in the kidney.

Conclusions

This method of capsule administration allows to deliver a small drug amount to the interest region without significant exposure on other vital organs, thereby reducing the drug side effects.

Acknowledgments

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EFFECTIVE DRUG DELIVERY TO THE RESPIRATORY PORTION OF THE LUNG USING SUBMICRON VATERITE PARTICLES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 81]

Introduction

High level of mortality caused by lung diseases is the great problem of our time. Complexity in the development of new drugs for the treatment of pulmonary diseases makes researchers to seek for new delivery systems, which can provide biocompatibility, prolonging time of release and possibly therapeutic effect inherently. Also the lungs can be considered as alternative route for drug delivery into the system.

Results and Discussion

The calcium carbonate particles were examined as the delivery system because of their high biocompatibility. The particles of calcium carbonate (vaterite) of different size (3.15 um, 1.35 um, 0.65 um) marked by conjugate of BSA and Cy7 were injected into the lungs of mice through tracheostome at a dose of 0.6 mg (volume of 60 µl). Vaterite particles were dispersed in sodium chloride buffer 0.9% at the concentration of 10 mg/ml. The biodistribution of calcium carbonate particles was observed during 72 hours using luminescence approach (In Vivo Imaging System). After sacrificed of mice the lungs, livers, kidney, spleens, stomachs were dissected and the images of these organs luminescence were made. The SEM images of lung cryo-slices with instillation of 0.6 um calcium carbonate particles were obtained. Furthermore, laser scanning confocal microscopy shows 0.65 um particles reaching the alveolar space. The delivery of fluorophore to the blood was assessed using Cy7 labeled 0.65 um particles. The pharmacokinetics of NIR fluorescence dye will be shown.

Conclusions

These studies establish that by using 0.65 um particles loaded with Cy7 we can efficiently access the respiratory portion of the lung, which represents a potentially efficient delivery mechanism for both the lung and the vasculature.

Acknowledgments

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OSTOGENIC POTENTIAL OF HUMAN ADIPOSE-DERIVED ASC52TELO CELL LINE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 82]

Introduction

Bone marrow stromal cells (BMSC) and cells from the stromal vascular fraction of adipose tissue (ASC) are now extensively studied for several tissue engineering approaches. In 2001 Zuk *et al.* described adipose-derived mesenchymal cells which could differentiate into multiple lineages, including osteoblasts [1]. Harvesting such cells from patients is less invasive compared to BMSC and results in less morbidity and higher amounts of osteoprogenitors in initial isolates and for further expansion in culture. Treatments of bone defects may include ASC instead of BMSC although ASC are reported to display lower osteogenic potential vs. BMSC [2]. Standard osteogenic inducers in culture include ascorbic acid phosphate, dexamethasone, β-glycerophosphate [3]. Bone morphogenetic proteins are known as potent osteogenic growth factors *in vivo* and *in vitro* and BMP-2 and -7 are already used in clinics [4]. In this study we have investigated the osteogenic potential of hTERT immortalized adipose derived mesenchymal stem cell line (ASC52telo, ATCC SCRC-400) that may serve as a useful model to study osteogenesis in adipose-derived MSC. In addition, given that several studies suggest that silencing BMP natural inhibitor Noggin may improve the process of differentiating stem cells towards osteoblast [5], we have investigated the role of Noggin in the osteogenesis of ASC52telo and other MSC of different than adipose tissue origin. This was important in the view of the latest finding that showed that Noggin binds to BMP receptors and it can induce osteogenic effects in osteoblastic cells [6].

Materials and Methods

ASC52telo cells were expanded in culture medium composed of 89% MEM Alpha, 10% FBS Q and 1% Penicilin-Streptomycin. Osteogenesis was induced with different combinations of dexamethasone (Dex), ascorbic acid phosphate (Asc), β-glycerophosphate (BGP) and bone morphogenetic protein-2 (BMP-2). Osteogenesis was evaluated by biochemical assay of cellular ALP activity at 7-day culture, the collagen and mineral staining at 14-day culture. All results were presented as means and SD. Statistical significant differences were assessed by one-way analysis of variance followed by Tukey's tests for multiple comparisons and $p<0.05$ was considered significant.

Results and Discussion

The highest increase in ALP activity in ASC52telo cells was observed after stimulation of cells with Asc and Dex. Notably, ALP activity levels after cell stimulation with Asc+Dex with and without 100 ng/ml BMP-2 were not significantly different. Dex seemed to play a key role in stimulation of ALP activity in ASC52telo cells (FIG. 1). Collagen synthesis was stimulated by Asc and Asc+BGP (FIG. 2). These results confirmed the crucial role of ASC in the synthesis of collagen by osteoblastic cells [3].

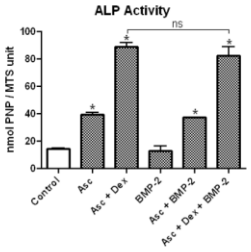

Fig 1. Alkaline phosphate activity after 7-d culture of ASC52telo cells with osteoinductive factors and BMP-2.

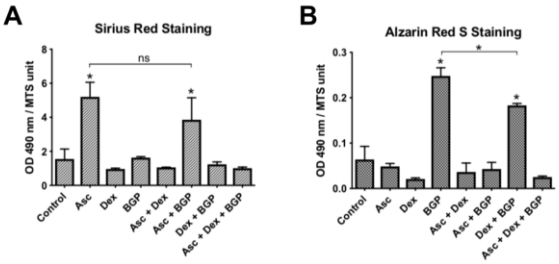

Fig 2. A - Collagen levels after 14-d culture of ASC52telo cells with osteoinductive factors. **B** - Mineralization levels after 14-d culture of ASC52telo cells with osteoinductive factors. *p<0,05, ns p>0,05.

On the other hand BGP was necessary for extracellular matrix mineralization, but it was effective alone or with Dex, but not together with Asc (FIG. 2). Interestingly enough, we have also found that Noggin increased ALP activity of BMSC cells treated with Asc+Dex and ongoing studies are aimed at examination of Noggin potential to induce osteogenesis in ASC52telo and other adult humans MSC.

Conclusions

Based on current results ASC52telo cell line shows good osteogenic potential in culture when stimulated with standard osteogenic inducers, i.e Asc, Dex and BGP, but these cells seem poorly responsive to rhBMP-2 similar to human BMSC [7]. Further studies will focus on in depth analyses of osteogenic markers in ASC52telo cell line and other than rhBMP-2 potential osteogenic growth factors.

Acknowledgments

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THE BIOLOGICAL ROLE OF CaO/SiO_2 RATIO AND P_2O_5 CONTENT IN SOL-GEL BIOACTIVE GLASSES INCORPORATED INTO PLGA

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[ENGINEERING OF BIOMATERIALS 153 (2019) 83]

Introduction

We have prepared composite materials obtained on the basis of PLGA copolymer and sol-gel derived bioactive glasses (BG). Such composites have been shown by us to display high biocompatibility and some of them can stimulate osteogenesis in human bone marrow stromal cells (hBMSC) [1-4]. The aim of this study was to examine to what extent the chemical composition of sol-gel derived bioactive glasses (SBG) incorporated at 50 w% to PLGA matrix affects early osteogenesis events and BMP signaling in BMSC. The ion dissolution products obtained from materials were examined for biological activity as well.

Materials and Methods

Eight SBGs with different CaO/SiO_2 ratios with and without P_2O_5 were incorporated at 50% to PLGA matrix and structured into thin films suitable for cell culture (TABLE 1).

TABLE 1. Chemical compositions of gel-derived glasses and their CaO/SiO_2 ratio.

Material	Chemical composition (%mol)			CaO/SiO_2
	SiO_2	CaO	P_2O_5	ratio
A1	40	60	-	1.50
T1	50	50	-	1.00
D1	60	40	-	0.67
S1	80	20	-	0.25
A2	40	54	6	1.35
T2	47	47	6	1.00
D2	60	36	4	0.60
S2	80	16	4	0.20

Human BMSC were harvested from iliac crest of adult patients (42-67 years old, both genders) according to the approved Institutional Review Board protocol (nr 1072.6120.254.2017). After isolation and expansion, BMSC were either seeded directly on the films or cultured on tissue culture plastic with condition medium (CM) harvested from the materials (FIG. 1). BMSC were examined for mRNA levels of Runx-2 and Osx at 2-day culture, BMP-2 and BMP-6, phospho-Smad1, 5, 8 and phospho-Tak1 at 3-day culture, alkaline phosphatase activity at 7-day BMSC cultures as well as for culture mineralization 21 days post cell seeding.

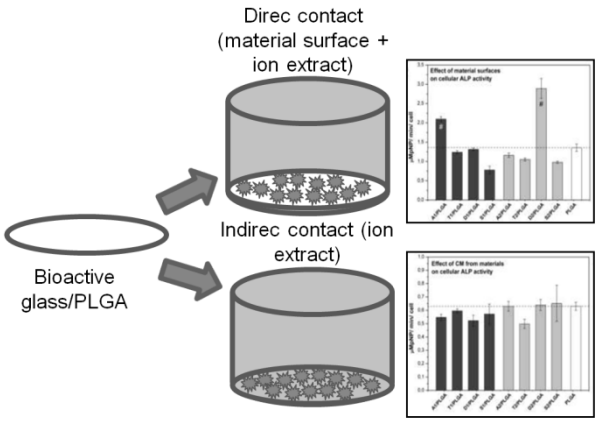

FIG. 1. General experimental scheme and representative results.

Results and Discussion

The materials without P_2O_5 stimulated higher RUNX-2 expression in BMSC and we observed higher mineralization level of the extracellular matrix in BMSC cultures grown in the presence of CM from these materials. In general, composites with higher content of CaO stimulated expression of OSX. CM from tested surfaces did not affect the activity of ALP, but culturing cells directly on some of the material surfaces resulted in ALP stimulation. P_2O_5 enriched materials stimulated the synthesis of BMP-2 and BMP-6 and the activation of Smad 1, 5, 8 proteins to a greater extent than these without P_2O_5. Moreover, most studied composite surfaces stimulated BMP-dependent TAK1 kinase.

Conclusions

Our studies confirmed osteoinductive properties of the studied composites although we have not found much correlation between the CaO/SiO_2 ratio in SBG, incorporated into PLGA, and BMSC response. The incorporation of P_2O_5 into SBG also played a significant role in a biological response. Furthermore, the examinations of ALP activity in cultures of human BMSC showed marked differences in cell behavior depending whether the cells were treated with culture medium conditioned with composite materials or they were directly seeded on the material surfaces. Although the ions released from some studied surfaces were sufficient to induce matrix mineralization in hBMSC, they were ineffective to activate cellular ALP activity in human BMSC. We believe the results obtained in this work may prompt further research regarding BMP pathways activation by composites that contain bioactive glasses as these pathways may be crucial to drive osteogenesis of BMSC on contact with bioactive materials. They also indicate the importance of studies dissecting the biological role of the ions released from bioactive materials and the role of the material surface itself.

Acknowledgments

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OSTEOGENIC POTENTIAL OF EXPERIMENTAL BIOACTIVE SURFACES IN BMP-RESPONSIVE MOUSE OSTEOBLASTIC CELLS AND HUMAN ADIPOSE DERIVED ASC52TELO CELL LINE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 84]

Introduction

We examined composite materials obtained from PLGA copolymer and sol-gel derived bioactive glasses (BG) (TABLE 1). Similar composites have been previously shown by us to display high biocompatibility and some of them can stimulate osteogenesis in human bone marrow stromal cells (BMSC) [3] [4] [6]. In this work, we studied these experimental surfaces in cultures of BMP-responsive mouse osteoblastic cells (BRITER, ATCC) and human adipose tissue derived ASC52telo cell line [8]. Bone morphogenetic proteins (BMPs), especially BMP-2 and -7 are clinically relevant and they are often used in tissue engineering approaches to deliver osteogenic growth factors. On the other hand, adipose-derived mesenchymal stem cells are known for their osteogenic potential although their ability to differentiate into osteoblasts is generally lower than that of BMSC [1] [2] [5] [7]. In this work BRITER cells with and without exogenous BMP-2 were either directly cultured on experimental surfaces or in the presence of condition medium (CM) harvested from the materials (FIG. 1). Similarly, human ASC52telo cells were either directly seeded on the material surfaces or cultured on tissue culture plastic in the presence of CM. Cells were stimulated with osteogenic inducers, including BMP-2 or 1,25(OH)2 VitD3. Osteogenesis was examined by the activity of BMP Response Element (BRE)-dependent Firefly Luciferase (FFLuc) in BRITER cells as well as alkaline phosphatase (ALP) activity in both BRITE and ASC52telo cell lines.

TABLE 1. Chemical composition of BGs incorporated at 50% into PLGA matrix.

SYMBOL	SiO_2 [%]	CaO [%]	P_2O_5 [%]
A1	40	60	-
D1	60	40	-
T1	50	50	-
S1	80	20	-
A2	40	54	6
D2	60	36	4
T2	47	47	6
S2	80	16	4
SiO_2	100	-	-

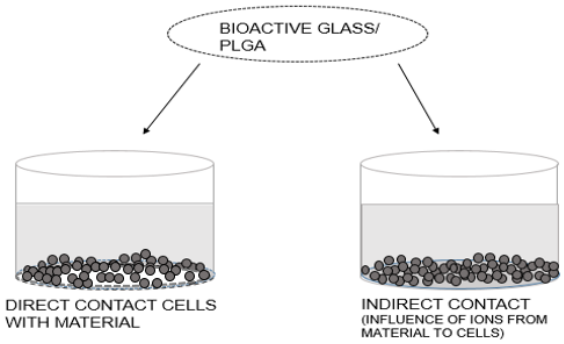

FIG. 1. Experimental scheme.

Materials and Methods

The activity of BMP Response Element (BRE)-dependent Firefly Luciferase (FFLuc) in BRITE cells was examined after 3-hour BMP-2 treatment (100 ng/ml) at day 3 culture, whereas ALP activity in BRITE cells was examined after 14-day culture with and without BMP-2. ASC52telo cells were examined for ALP activity at day 10 culture.

Results and Discussion

BRITER cell treated with BMP-2 significantly increased Luciferase activity in cells grown directly on high-silica materials, but ALP activity was not induced for these materials. Importantly, BMP-2 treatment of cells grown on TCP in the presence of CM harvested from experimental surfaces showed different results and the cells significantly increased Luciferase activity when treated with CM from high-calcium composites. ASC52telo treated with BMP-2 significantly increased ALP activity in cells grown on high-silica material whereas treatment with VitD3 was ineffective to induce ALP activity in any material type except for pure SiO_2 and PLGA. Treatment of ASC52telo cells with CM from experimental surfaces was ineffective to induce alkaline phosphatase activity despite the presence of osteogenic inducers.

Conclusions

Our results indicate important differences in cell response to the experimental surface depending on the cells type as well on the general osteogenic conditions. This is important especially for bone tissue engineering approaches aiming to deliver biomaterial constructs loaded with potentially osteogenic cells. Also, our results indicate the important differences in biological effects obtained with cells grown directly on the material surfaces vs. cells growth in the presence of material dissolution products. It may have important implications regarding the in vivo biomaterial implantation as cell-loaded biomaterials may provide different biological results vs. empty biomaterial scaffolds.

Acknowledgments

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THE EFFECT OF THIN FILMS MADE OF CHITOSAN/COLLAGEN, POTASSIUM SILICATE AND TANNIC ACID ON VIABILITY OF CANCER AND HEALTHY CELLS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 85]

Introduction

Composites consisting of a matrix and structural component are a promising and quickly-expanding branch of materials and tissue engineering. Current ecological issues call out for changes toward more natural lifestyle and this trend is also observed within the field of biocomposites – composite materials in which at least one component comes from the natural origin [1]. There is also substantial need of the medical market for innovative and natural biocomposites serving as drug and cell delivery vehicles and/or medical implants. This study was carried out with the composites prepared with the chitosan and collagen matrix supplemented with potassium silicate as an inorganic component. The latter was added to improve material properties. In addition, a small amount of tannic acid was added to stabilize polymers. The obtained composite materials were tested in human cancer cell lines: MNT-1 (highly pigmented melanoma), SK-MEL-28 (malignant melanoma) and Saos-2 (osteosarcoma) as well as in healthy cells: HaCaT (immortalized keratinocytes) and human bone marrow stromal cells (BMSC).

Materials and Methods

Collagen (COLL), chitosan (CTS) and tannic acid (TA) were dissolved separately in 0.1M acetic acid, first two at 1% concentration and the last one at 2%. COLL and CTS were mixed at 50/50 wt/wt% ratio. Then, 5 and 20 wt% of 2% TA was added followed by supplementation with 5 and 10 wt% of potassium silicate (PS). Polymer solution with inorganic additive was stirred to obtain a homogeneous mixture. Then it was placed in 24-well cell culture plates for 48h to form films after solvent evaporation. The films were sterilized for 10 minutes in 70% ethanol (water solution) and then rinsed with PBS. All types of cells used in this study were seeded directly on the material's surface in serum-containing media at a density of $1x10^4/cm^2$. Culture media were exchanged on day 2 culture. On day 6 culture, CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) was performed to assess cells viability, accordingly to the manufacturer's protocol. Values of MTS absorbance (λ = 492 nm) were averaged and recalculated to a percent change in the metabolic activity of cells on the surfaces consisting of chitosan, collagen and either 5% or 10% potassium silicate and tannic acid vs. cell viability on the materials without tannic acid (assumed as 100%). Results were statistically analyzed with one-way ANOVA and post-hoc Tukey; $p<0.05$ was considered significant.

Results and Discussion

TABLE 1. Cell metabolic activities (viabilities) are presented for MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. Asterisk (*) stands for statistically significant results vs. material composed of CTS/COLL 50/50 wt/wt% ratio with either 5 or 10% PS without TA (assumed as 100% viability).

	5% PS 5% TA	**5% PS 20% TA**	**10% PS 5% TA**	**10% PS 20% TA**
MNT-1	* 51% ± 5%	* 128% ± 17%	* 51% ± 7%	* 64% ± 4%
SK-MEL-28	105% ± 5%	* 157% ± 13%	107% ± 3%	* 145% ± 5%
Saos-2	104% ± 1%	* 231% ± 13%	86% ± 4%	* 190% ± 13%
HaCaT	82% ± 6%	137% ± 22%	81% ± 5%	135% ± 19%
BMSC	133% ± 18%	* 294% ± 18%	102% ± 16%	* 219% ± 24%

Significant decrease in viability (up to 50%), was observed for MNT-1 cells on materials containing 5 or 10% PS and 5% TA. The addition of 20% TA to 5% PS materials increased the viability of all cell types except HaCaT, in which no difference was observed. BMSC cells showed the highest, three-fold increase in the viability on materials containing 5% PS and 20% TA. 20% TA added to 10% PS materials caused an increase in the viability of all cell types except MNT-1, where this addition caused a decrease in viability.

Conclusions

The addition of inorganic component – potassium silicate (PS) and tannic acid (TA) to thin films composed of chitosan and collagen affects cell viability of MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. The most significant changes in cell viability were observed for cells cultured on composites prepared with either 5 or 10% PS and 20% TA. Considering the cancer field, it is of interest that the materials tested in this study showed a significant decrease of MNT-1 (melanoma) viability. The apoptotic effect of tannic acid (TA) on estrogen receptor-positive cancer cells has been previously shown [3] and melanoma cells have been shown to express estrogen receptors [4]. This preliminary assessment of PS and TA supplemented chitosan/collagen-based materials suggests they may display anti-cancer properties but further research is required to verify this thesis.

Acknowledgments

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THE FACTORS GOVERNING HYDROLYTIC DEGRADATION OF POLYESTERS-BASED SCAFFOLDS IN BONE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 153 (2019) 86]

Introduction

Degradation of the tissue-engineered constructs is considered to be one of the main subjects of interest in case of designing and fabricating polymeric scaffolds. Degradation rate of such scaffolds should, in this case, follow a strictly defined pathway, in which tissue formation should be preceded by gradual degradation while ensuring certain mechanical support [1]. There are many factors, which in fact can affect the degradation kinetics of the constructs fabricated via additive manufacturing. One of them are certainly structural properties of the constructs, which for instance, relate to adopted architecture of the scaffold. Many studies demonstrated that degradation rate can be also easily modified by using different materials and their composition [2,3]. As some of internal features controlling the degradation kinetics was above stated, it is worth mentioning that the external factors could also play an important role in accelerating degradation rate, especially in the laboratory conditions. Instead of conducting time-consuming and tedious *in vitro* degradation of long-time degrading constructs, new methods of accelerating their degradation kinetics has been proposed. One of them pertain to the static and dynamic medium systems, in which degradation occurs slower in case of dynamic environment [4]. The degradation can be also accelerated by conducting the experiment in the elevated temperatures[5]. But it is worth considering wheatear the properties of the material will not be affected and the scaffolds degradation will not be falsified in any way.

As such we would like to communicate the topic of scaffold's degradation and by presenting some of the methods of accelerating it provides a special insight in the broad topic of manipulating the degradation kinetics.

Materials and Methods

The L-lactide-co-glycolide (PLGA), L-lactide-co-*ε*-caprolactone (PLCL) and their tricalcium-phosphate-loaded (TCP) composites containing 20 and 40 wt% of filler were fabricated using modified fused deposition modeling. We designed scaffolds with filament lay-down pattern of 0°/90° and with or without the modifications of filament distance in n+2 layer, shifted and non-shifted constructs were obtained, respectively. To investigate the effect of the temperature on degradation profile, we conducted two separate degradation experiments, dynamic and static, in which the change of mass, pH, water absorption and initial molecular weight loss (M_{w0}) was detected in phosphate buffered saline (PBS) at 37°C for up to 48 weeks and 50°C for up to 3 weeks, respectively. Subsequently, the effect of architecture modifications and its effect on degradation behaviour was explained utilizing fluid flow simulations. The scaffolds morphology was evaluated utilizing scanning electron microscopy (SEM) and the visualization of the topography was performed utilizing atomic force microscopy (AFM). Surface area to volume ratio (SVR) and porosity were determined using micro-computed tomography (μCT). Thermogravimetric measurements were used to assess the characteristic temperatures for investigated materials.

Results and Discussion

Architecture

In the case of dynamic and static degradation, accelerated degradation was observed in the case of shifted constructs. We assume that faster degradation may be attribute to their tortuosity, making them less permeable in which accumulation of acidic products in the tortuous architecture is observed.

Static and dynamic conditions

The degradation of PLGA- and PLCL-based scaffolds proceeded faster in case of static conditions. We observed a decrease of M_{w0} in the shorter time comparing to dynamic conditions at 37°C. Our findings agree with other studies, where degradation rate was reduced in case of scaffolds undergoing dynamic degradation, in which continual removal of acidic degradation products was observed.

Degradation at elevated temperatures

The rapid decrease of initial Mn and Mw was observed during 3 weeks of degradation at 50°C. Interestingly, PLGA-based scaffolds as the first started to crumble and disintegrate, contrary to PLCL-based ones. However, a different situation was observed at 37°C, where PLGA-based samples sustained mechanical integrity up to 24 weeks. It is believed that glass transition temperature (T_g) of polymer determines the behaviour of polymeric chains during various experimental conditions. Degradation performed below material's T_g will be dependent on mobility of polymer chains, which in this case is essentially zero. Thus performing degradation at elevated temperatures is not always suitable for certain materials.

Conclusions

There is a need to develop standard protocols for assessing accelerated polyester-based scaffold degradation. However, the establishment of such protocols is challenging due to complexity of factors accounting hydrolysis. In the present study, we investigated some factors leading to accelerated degradation, pointing that not only architecture, materials' properties but also degradation conditions can change the degradation rate of polyester-based scaffolds.

Acknowledgments

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SYNTHESIS AND CHARACTERIZATION OF NOVEL AMPHIPHILIC POLY(2-OXAZOLINE)-BASED COPOLYMERS FOR SURFACE MODIFICATION OF BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 87]

Introduction

Poly(2-oxazolines) (POx) are an important class of polymers that have attracted substantial attention recently and are emerging as stabilizers, adhesives, surfactants and dispersants. Polymer synthesis from 2-oxazoline monomers is typically carried out via a cationic ring opening polymerization [1].
The aim of this study was to synthesize novel gradient amphiphilic poly(2-oxazoline)-based copolymers containing reactive allyl groups for further modification e.g. by peptides or drugs. To this end POx containing free allyl groups with different ratio of 2-ethyl-2-oxazoline (EtOx) to 2-(4-allyloxyphenyl)-2-oxazoline (AllPOx) were prepared.

Materials and Methods

AllPOx and methyl 4-nitrobenzenesulfonate were dried in a flask for 3 h. Then, EtOx and benzonitrile (2 mL) were added. Copolymerization was performed for 24 h at 110°C under argon and terminated with methanolic KOH (0.1 mol/dm^3, 1.2 mL) at room temperature for 2 h.
Gradient copolymers of EtOx with AllPOx were prepared in different molar ratios of both monomers (90:10, 80:20, 85:15, 75:25, 70:30 and 60:40) through one-pot cationic copolymerization at 110°C for 24 h in benzonitrile (c = 3 mol/dm^3). In all cases, cationic copolymerizations were initiated by methyl 4-nitrobenzenesulfonate ([M]/[I]=100). Prepared copolymers were purified by dialysis against ethanol, water/ethanol 70:30, and water.
The ^{1}H NMR spectra were recorded in $CDCl_3$ at room temperature on a Varian VXR-400 using tetramethylsilane (TMS) as an internal standard. FTIR (Nicolet iS5-iD5spectrometer with the resolution of 4 cm^{-1}), in attenuated total reflection mode (ATR, with ZnSe crystal) was used. XPS studies were performed on K-Alpha high-performance Thermo Fisher spectrometer to study surface chemical composition. To evaluate molar mass and dispersity GPC was used (Agilent Technologies, trifluoroethanol, PMMA calibration).
Cytotoxicity of the obtained POx was assessed in contact with mice 3T3 fibroblast cell line by MTT assay at 560 nm and 750 nm.

Results and Discussion

Gradient copolymers of 2-ethyl-2-oxazoline (EtOx) with 2-(4-allyloxyphenyl)-2-oxazoline (AllOPhOx) were prepared in different molar ratios of both monomers through one-pot cationic copolymerization (FIG. 1).

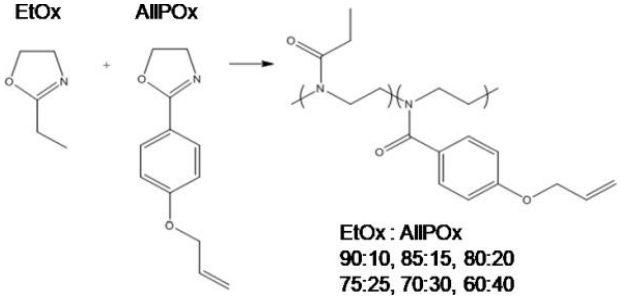

FIG. 1. Synthesis of poly(2-oxazolines) with different ratio of EtOx:AllPOx.

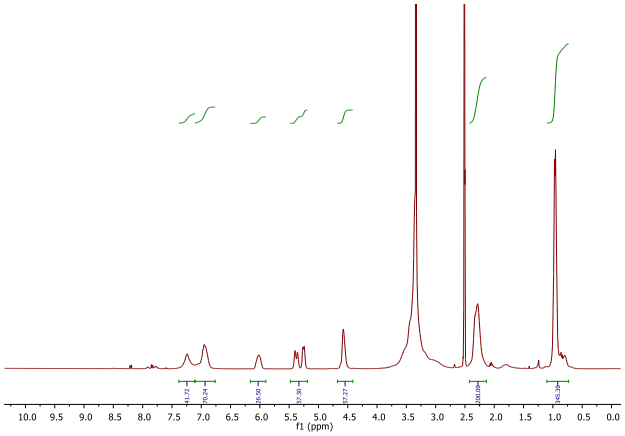

FIG. 2. ^{1}H NMR spectrum of POx with EtOx:AllPOx 80:20.

^{1}H NMR confirmed the composition of copolymers to be very close to feeding ratio of monomers (e.g. 78:22 for EtOx:AllPOx feeding ratio of 80:20). XPS showed presence of carbon, oxygen and nitrogen on the surface; atomic ratio was as expected. FTIR results showed the characteristic bands for POx: at 1130 cm^{-1} and 1620 cm^{-1}; the latter is characteristic for poly(2-oxazolines) and is due to stretching vibrations of C=O and C-N groups.
Molar mass for EtOx:AllPOx feeding ratio of 80:20 was 9700 g/mol, D=1.63. Obtained POx were found cytocompatible with model cells.

Conclusions

In this study, we obtained library of POx with defined structure and surface properties suitable for modification with peptides supporting cell adhesion.

Acknowledgments

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HYDROGEL MODIFICATION WITH THE USE OF $CaCO_3$ MICROPARTICLES ENRICHED WITH ANTIBACTERIAL PEPTIDES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 88]

Introduction

Antibiotics have been largely produced and overused in many countries. This led to an increase of the resistance of bacteria and biofilms to commonly used antimicrobial agents [1]. Novel methods for efficient eradication of bacterial infections are needed. Antibacterial peptides (ABPs) – cationic molecules consisting of 12-45 amino acids, are promising alternatives for antibiotics. ABPs are able to hamper bacterial proliferation as effectively as antibiotics, whereby bacteria are not able to develop resistance for ABPs [2]. The aim of the present study was to fabricate ABPs-enriched calcium carbonate ($CaCO_3$) microparticles (MPs) and use them for modification of gellan gum (GG) hydrogel in order to develop an injectable composite material that can be used for the treatment of bacterial infections in bones. Bacitracin (BCT) was used as a model ABP.

Materials and Methods

$CaCO_3$ MPs were fabricated using co-precipitation method. In brief, 5 ml of aqueous 0.3M $CaCl_2$ solution was mixed with 5 ml of aqueous 0.3M Na_2CO_3 solution under magnetic stirring (1000 rpm, 30 s). For the preparation of ABPs-enriched MPs, BCT was added to Na_2CO_3 solution at different concentration ranging from 0 (control) to 2 mg/ml. The MPs were fabricated in the same way as unloaded MPs. Following the precipitation procedure, MPs were collected and purified using repetitive centrifugation (5000 rpm, 5 min) and rinsing with deionized water (3x). Finally, MPs were air dried at 37°C overnight. MP morphology was evaluated under scanning electron microscopy (SEM) and BCT loading efficacy was determined using bicinchoninic acid (BCA) assay.
GG was dissolved in water at 90°C at a concentration of 1.11% (w/v) and then cooled down to 60°C. Then 9 ml of GG solution was mixed with 0.5 ml of aqueous suspension of MPs (0-200 mg/ml giving final concentration of MPs of 0-2%, unloaded or loaded with BCT) and 0.5 ml of aqueous $CaCl_2$ solution (0.3%, cross-linking agent). A mixture was cast into a Petri dish (for mechanical testing, SEM analysis and *in vitro* testing) or inserted into 2 ml syringes (for injectability testing) and cooled down to 4°C. The morphology, Young's modulus, injectability and chemical composition (FTIR spectroscopy) of the samples were analysed together with *in vitro* performance of the materials in contact with MG-63 osteoblast-like cells.

Results and Discussion

Obtained MPs were highly porous and spherical in shape with the average particles size ranging from 2.4 – 5.9 µm (FIG. 1). BCT encapsulation efficiency (EE) was 18 – 25%, resulting in BCT loading in MPs between 4.6 to 44.4 µg BCT per 1 mg of MPs.

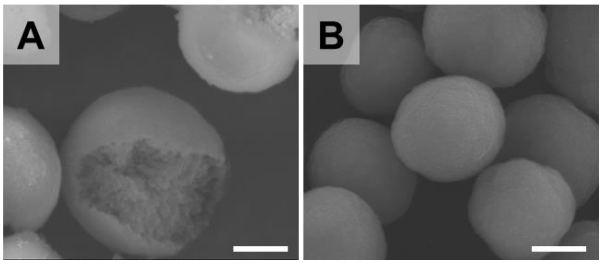

FIG. 1. – SEM images of $CaCO_3$ microparticles: unloaded (A) and loaded with BCT (B). Scale bar: 2 µm.

MPs were successfully loaded into GG hydrogels as confirmed by both SEM/EDX (FIG. 2) and FTIR spectroscopy. The MPs were distributed uniformly across the whole volume of GG and no signs of MP agglomeration were observed.

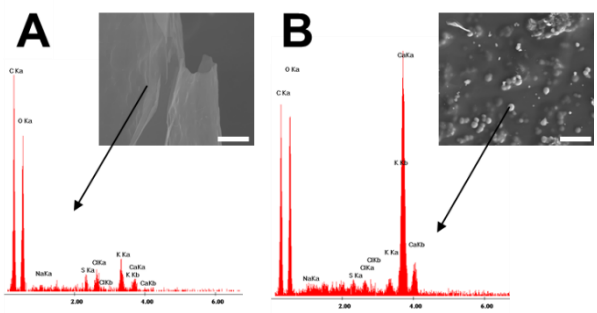

FIG. 2. SEM images (scale bar: 20 µm) and EDX analysis of GG: without MPs (A) and with 1% MPs (B).

The addition of up to 1% of MPs did not significantly influence mechanical properties of GG hydrogels (both in terms of Young's modulus and injectability). Also no differences were observed in the case of GG samples loaded with BCT-enriched MPs.
In vitro studies of GG-MPs composites showed increased proliferation of MG-63 cells in the presence of at least 0.25% of MPs in GG (FIG. 3).

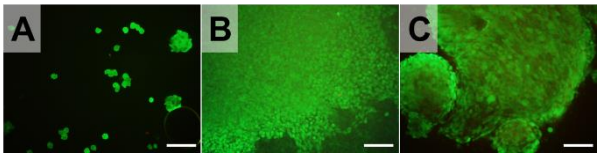

FIG. 3. Live/dead staining of MG-63 cells cultured for 14 days on GG-MP composites: without MPs (A), with 0.5% MPs (B) and with 1% of MPs (C). Scale bar: 100 µm.

Conclusions

Co-precipitation method allowed for fabrication of uniform spherical $CaCO_3$ MPs enriched with an exemplary ABP – bacitracin. MPs can be incorporated into GG hydrogel and addition of up to 1% of MPs does not decrease mechanical properties or injectability of GG, but it significantly improve *in vitro* performance of GG. Further studies will focus on evaluation of antibacterial properties of the developed materials.

Acknowledgments

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THE STUDY OF POLY(ε-CAPROLACTONE)-BASED POLYURETHANES FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 89]

Introduction

Polyurethanes are a class of polymers composed mainly of isocyanates, polyol and chain extender units linked by urethane groups. Functionalization of polyurethanes through the addition of natural polysaccharides like chitosan has been performed to enhance biomedical applications of these polymers. Poly(ε-caprolactone) (PCL) is classified as a semicrystalline polymer characterized by melting temperature approximately 60°C and glass transition under room temperature [1].
Janik and co-workers reported successful modification of PU based on IPDI, PCL, and BDO via incorporation of chitosan. The inclusion of chitosan as a crosslinker has yielded polymer stable under sterilization conditions [2]. This solution offers materials suitable for heart valves. Mahmood et al. [3] have proposed the synthesis of chitosan-based polyurethane elastomers involving 2,4-toluene diisocyanate (TDI) and poly(ε-caprolactone) (PCL). Accordingly, chitosan-based polyurethane may link the properties of both components - chitosan and polyurethane.
The objective of the study is to demonstrate as a proof of concept an application of PCL as soft segments instead of PEG-2000. Moreover, we have used chitosan as a chain extender and hydroxyapatite to improve the bioactive character of polymer.

Materials and Methods

Poly(ε-caprolactone)-based polyurethane were synthesized using chitosan as a chain extender and hydroxyapatite as filler to enhance biocompatibility. The polyurethane prepolymer was prepared by the polymerization reaction of 1,6-hexamethylene diisocyanate (HDI) with poly(ε-caprolactone) (PCL) diol using dibutyltin dilaurate (DBTDL) as a catalyst. The synthesis was carried out under nitrogen atmosphere at 60°C. The bulk polymerization method was chosen as the synthetic way as it allows to avoid toxic solvents. The crosslinking process occurred via the reaction of urethane prepolymer with butanediol (BDO) and chitosan. The crosslinking process was conducted at 60°C for 24 hours.
Differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) have been applied to determine the thermal properties of the PCL-based polyurethane. The Fourier transform infrared spectroscopy (FTIR) analyses were carried out to analyse the structure of PUs. The dispersion of hydroxyapatite in the polyurethane matrix was examined by scanning electron microscopy (SEM).

Results and Discussion

TG curves showed three-step degradation of polyurethane – FIG. 1.

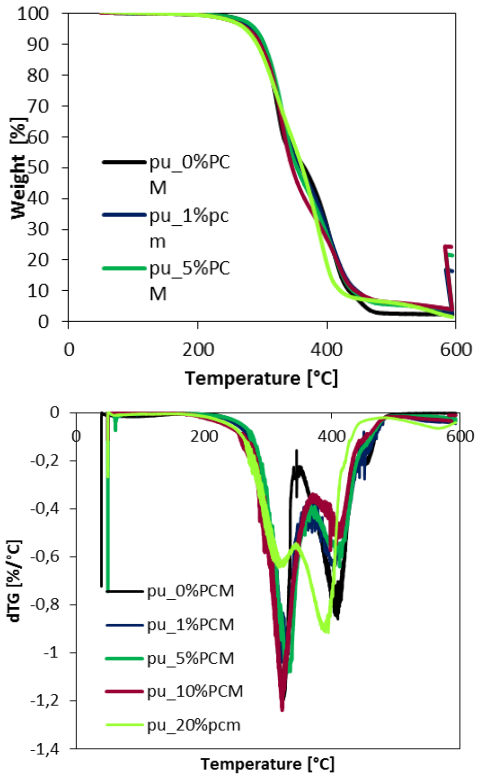

FIG. 1. The TG curves for polyuerthane samples.

The first one is associated with degradation of the hard segment of isocyanates. The second step is attributed to the degradation of polyol units. The third corresponds to the degradation of organic residues. The incorporation of PCL instead of PEG 2000 causes a decrease in the thermal stability.
The DSC profile showed a glass transition of soft and hard segments and there was an endothermic peak for melting of polyol units. The FTIR study confirms the completion of the polymerization reaction and crosslinking process of the obtained prepolymer. The SEM studies show the high degree of dispersity of hydroxyapatite in the polymer matrix.

Conclusions

FTIR analysis indicated the occurrence of the urethane bonds. The SEM photographs show good dispersion of hydroxyapatite in a polymer matrix. Thermal analysis techniques reveal that incorporation of PCL leads to a decrease in PU thermal stability.

Acknowledgments

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ACETAL POLYMERS MODIFIED WITH HYBRID INORGANIC-ORGANIC HYDROXYAPATITE-BASED SYSTEMS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 90]

Introduction

Acetal polymers, also known as polyoxymethylene (POM) or polyacetals, are formaldehyde-based engineering thermoplastic polymers apply in a broad range of applications because of their improved properties [1]. They have been used in different areas including automotive and mechanical industry, electronics, consumer goods but also in the production of elements of medical devices and drug delivery systems [2-3]. However, the using of POM as biomaterial is limited due to a lack of bioactivity and poor thermal stability. Considering the upper limitations, the composites made of POM matrix and the hybrid, inorganic-organic hydroxyapatite-based filler (HAp-*g*-PEG) with improved thermal stability, bioactivity and the hybrid filler dispersion in POM matrix, were prepared and investigated in this study.

Materials and Methods

POM copolymer (POM_C, Ultraform®, BASF) and POM homopolymer (POM_H, Delrin®, DuPont) were used as a composites matrix. Hydroxyapatite (HAp, nGimat) in the shape of nanopowder was functionalized with poly(ethylene glycol) (PEG, Sigma Aldrich) of three average molar mass of 600, 2000 and 6000. 1,6-hexamethylene diisocyanate (HDI) was used as a coupling agent. In consequence, the hybrid, inorganic-organic fillers of HAp-HDI-PEG (HAp-*g*-PEG) intended to POM composites were synthesized.

The dispersion of fillers in the polymer matrix was observed using transmission electron microscopy (TEM). Thermal degradation, the kinetics of thermal degradation and gaseous degradation products of composites were analyzed using thermogravimetry (TG), Friedman method and multiple non-linear regression (Netzsch Thermokinetics). Identification of degradation products was performed using TG-FTIR/MS technique. Bioactivity of composites was confirmed by scanning electron microscope (SEM) with energy dispersive X-ray spectroscopy (EDX) after incubation in simulated body fluid (SBF).

Results and Discussion

The TEM micrographs of POM_C and POM_C/HAp-*g*-PEG composites are shown in FIG. 1. The hybrid HAp-*g*-PEG particles, in the form of connected nanospheres, are well dispersed in the POM matrix. This is possible because a hybrid material exhibits a higher affinity to polymer matrix in comparison to inorganic nanoparticles.

The mechanism and kinetics of thermal degradation investigations of POM/HAp-*g*-PEG materials shown a strong increase in thermal stability. For pristine POM, one-stage degradation mechanism with autocatalysis was observed. While for composite materials, an additional of n-th order reactions or *n*-dimensional nucleation (Avrami–Erofeev equation) were observed.

From TG-FTIR/MS results, it was found that the main degradation product for POM and POM/HAp-*g*-PEG composites is formaldehyde and the amount of other degradation products is lower in comparison to unmodified POM. This method also confirmed the thermal stability increase in comparison to pure POM.

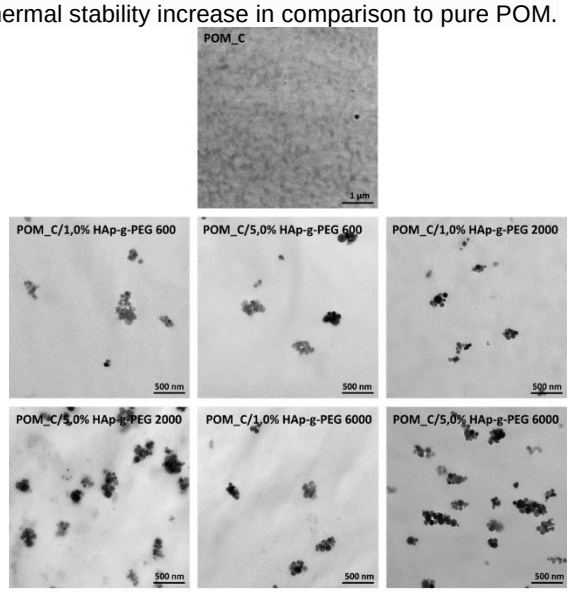

FIG. 1. TEM micrographs of POM_C/HAp-*g*-PEG composites.

As can be seen in FIG. 2, a thick layer of apatite was formed on the POM composites surface after 21 days incubation in SBF. It confirmed that POM/HAp-*g*-PEG materials have a great potential for the *in vivo* bone bioactivity.

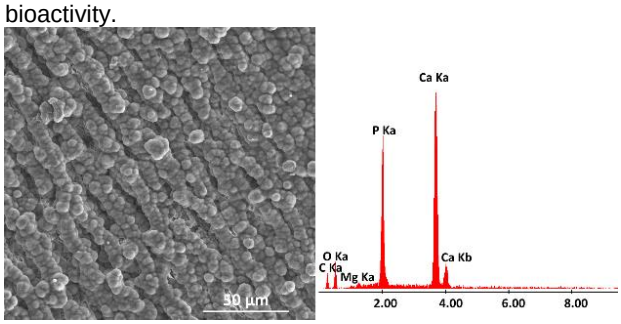

FIG. 2. SEM/EDX result for POM_C/5,0% HAp-*g*-PEG 2000 after incubation in SBF.

Conclusions

The chemical modification of HAp nanoparticles with PEG provides good, homogenous dispersion of filler within the POM matrix. The HAp-*g*-PEG additives affected the POM degradation mechanisms, as a result the thermal stability of POM/HAp-*g*-PEG composites was improved. The presence of HAp particles in HAp-*g*-PEG additive improved bioactivity of composites that can be considered for orthopedic applications.

Acknowledgments

Authors are grateful to the Polish National Science Centre for financial support under the Contract No. 2016/21/B/ST8/00449.

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ELECTROCHEMICAL TESTS OF CoCr ALLOY MADE WITH ADDITIVE TECHNOLOGY

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[ENGINEERING OF BIOMATERIALS 153 (2019) 91]

Introduction

One of the methods of treatment of missing human teeth without the necessity of the placement of dental implants is the use of a metal frame removable partial dentures. It allows to restore the aesthetics and functionality of the stomatognathic system to patients who, among others they feel anxiety about implantation or are facing bone atrophy. The use of a prosthesis also significantly reduces the cost of treatment compared to implants [1]. The introduction of 3D printing technology has caused this method to be applicable also in the manufacture of frames [2]. Although frames made with this technology are better suited to the patient's anatomical features, the method of surface preparation is still a challenge, if only because of their complicated shape. When designing the final form of the surface layer for this type of products made using 3D printing, it is necessary to take into account, first of all, the morphology of the substrate, as well as the conditions in which it will function in the human mouth [3].

Materials and Methods

CoCr alloy samples made using the classic technique (casting) and using additive technology were selected for the tests - 3D printing after electrochemical polishing with parameters that allow obtaining a surface roughness Ra <0.3 μm. The potentiodynamic tests were carried out as recommended by the ASTM F2129 standard. Potentiodynamic studies were performed using a potentiostat PGP-201 Radiometer Analytical SAS. As reference electrode a saturated calomel electrode NEK KP-113 was used, and as the auxiliary electrode - platinum PTP-201. To determine the values characterizing the corrosion resistance of tested samples, Stern method was applied. EIS measurements were performed using a potentiostat AutoLab PGSTAT 302N along with a set of electrodes provided with the module FRA2 (Frequency Response Analyser). The measuring system that was used during the study enables research in the frequency range 10^4 ÷ 10^{-3}Hz. The voltage amplitude of the sinusoidal excitation signal was 10 mV.
The tests were carried out in artificial saliva at a temperature of T = 37 ± 1°C.

Results and Discussion

The examples of polarization curves recorded for the CoCr alloy are shown in FIG. 1. The values of corrosion potential, polarization resistance and transpassivation potential determined on their basis clearly showed better pitting corrosion resistance of the CoCr alloy made by 3D printing - TABLE 1.
In turn, the tests carried out using electrochemical impedance spectroscopy confirmed the results obtained in potentiodynamic tests. On the surface of the CoCr alloy made by 3D printing, the presence of a double layer with better electrochemical properties in relation to the oxide layer formed on the surface of the cast CoCr alloy was shown - TABLE 2.

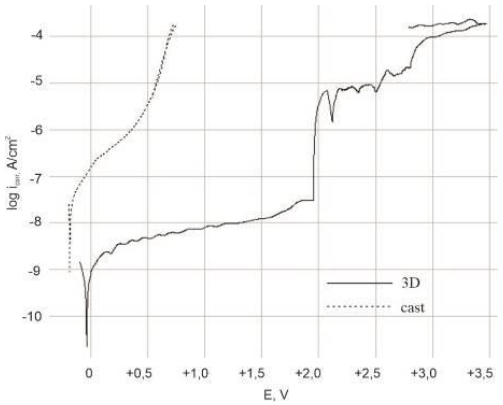

FIG. 1. Examples of polarization curves for CoCr alloy.

TABLE 2. Results of potentiodynamic test.

CoCr alloy	E_{corr}, mV	E_{tr}, mV	Rp, kΩcm^2
3D printing	-32	+1 950	3 081
cast	-189	+650	53

The best fit of the model spectra to the impedance spectra determined experimentally in artificial saliva is provided by the circuits shown in FIG. 2.

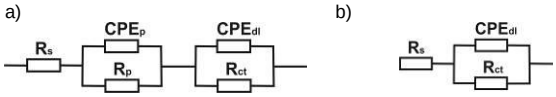

FIG. 2. Electrical model of equivalent circuit for CoCr alloy – artificial saliva: a) 3D printing, b) cast.

TABLE 2. Results of EIS.

CoCr alloy	R_s, Ω·cm^2	R_p, kΩ·cm^2	CPE_p		R_{ct}, kΩ·cm^2	CPE_{dl}	
			Y_0, $\Omega^{-1}cm^{-2}s^{-n}$	n		Y_0, $\Omega^{-1}cm^{-2}s^{-n}$	n
3D printing	53	201	0,2121E-4	0,9	1 908	0,2367E-4	0,9
cast	52	-	-	-	655	0,8984E-4	0,9

Conclusions

Based on the conducted electro-chemical tests, it was found that 3D printing technology is a more preferred method of manufacturing a metal frame of dentures made of CoCr alloy. Corrosion resistance in the environment of artificial saliva for this type of technology is higher in comparison with products made of CoCr alloy using classic technology (casting). In addition, proposing suitable surface treatment variants for CoCr alloy products made with this technology is of prospective importance and will contribute to the development of technological conditions that allow obtaining antibacterial and anti-fungal coatings.

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THE PREPARATION AND CHARACTERIZATION OF MICROPARTICLES BASED ON WHEY PROTEIN ISOLATE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 92]

Introduction

Whey protein isolate (WPI) is a by-product of the dairy industry obtained during the industrial production of cheese or casein. It consists of a mixture of globular proteins, mainly β-lactoglobulin and α-lactoglobulin. Due to its excellent gelling, film-forming and emulsifying properties, WPI is widely used in various food products. Recent studies show that WPI can also be a matrix for encapsulation of biologically active compounds. Many techniques have been developed for the production of protein-based microparticles including spray drying, coacervation or emulsion-crosslinking method [1-5]. However, these methods require heating or using organic solvents in at least one stage of production, which can negatively affect encapsulated active ingredients. For this reason, there is a need to develop a method for preparing WPI-based microparticles, which allow protection and stability of the incorporated substance.
The aim of this work was to prepare and characterize microparticles based on whey protein isolate and sodium alginate containing *Calendula officinalis* flower extract.

Materials and Methods

In order to obtain WPI-based microparticles, aqueous solutions of whey protein isolate and sodium alginate (ALG) were mixed with *Calendula officinalis* flower extract and transferred to the encapsulator's pressure bottle. A large flat beaker filled with 2% (w/v) calcium chloride solution was placed under a nozzle on a magnetic stirrer. The diameter of the nozzle, as well as parameters of the encapsulator (pressure, liquid flow, vibration frequency and electrostatic voltage) were set to obtain the correct microparticle chain in the light of a stroboscope lamp.

Composition of microparticles:

- 4% (w/v) WPI aqueous solution + 0.5% (w/v) ALG aqueous solution + 0.5% *Calendula officinalis* flower extract → **WPI 4% ALG 0.5%**
- 4% (w/v) WPI aqueous solution + 1% (w/v) ALG aqueous solution + 0.5% *Calendula officinalis* flower extract → **WPI 4% ALG 1%**
- 5% (w/v) WPI aqueous solution + 0.5% (w/v) ALG aqueous solution + 0.5% *Calendula officinalis* flower extract → **WPI 5% ALG 0.5%**
- 5% (w/v) WPI aqueous solution + 1% (w/v) ALG aqueous solution + 0.5% *Calendula officinalis* flower extract → **WPI 5% ALG 1%**

The morphology and size of obtained microparticles were determined using a stereo microscope Motic SMZ-171 BLED. Swelling properties was measured by weighing the wet microparticles and after drying for 24h at room temperature. Loading capacity of active substance was performed using UV/Vis spectrophotometric method.

Results and Discussion

Microparticles based on WPI and sodium alginate have regular, spherical shape and rough surface (FIG. 1). The prepared microparticles are homogeneous and have very similar dimensions - the standard deviation of their average size is less than 2.8% of the particle size. Their diameters after drying have significantly decreased. The largest difference in their sizes before and after drying was observed for microparticles consisting of 5% WPI and 0.5% ALG (2339±56 μm and 982±16 μm, respectively). WPI-based microparticles have a high swelling ability ranging between 670–830%. The lowest swelling degree was observed for WPI 4% ALG 1% microparticles, while the greatest swelling capacity have WPI 5% ALG 0.5% microparticles. Similar dependencies were also observed for loading capacity. The lowest amount of *Calendula officinalis* flower extract was loaded into WPI 4% ALG 1% microparticles (approx 170 mg/g). The most effective incorporation of extract was noted for microparticles composed of WPI 5% ALG 0.5% (approx 290 mg/g).

FIG. 1. Microscope images of WPI and ALG microparticles containing active substance: A) swollen microparticles, B) dry microparticles.

Conclusions

The obtained spherical microparticles based on whey protein isolate and sodium alginate have successfully incorporated *Calendula officinalis* flower extract. Higher WPI concentration and lower sodium alginate content contributed to the incorporation of a higher amount of *Calendula officinalis* flower extract into the microparticles. Due to biodegradability and occurrence in nature of WPI and sodium alginate, the developed microparticles may be the basis for obtaining a new class of materials for cosmetic, dermatological, pharmaceutical and biomedical purposes.

Acknowledgments

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HYDROGEL SCAFFOLDS FOR MEDICAL APPLICATIONS OBTAINED BY INDIRECT 3D-PRINTING METHOD

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[ENGINEERING OF BIOMATERIALS 153 (2019) 93]

Introduction

Hydrogels are more and more widely used in biomedical applications, e.g. as dressings and matrices for tissue and organ engineering [1]. Classical direct hydrogel printing is challenging, particularly for fast gelling hydrogels. Indirect 3D printing method involves material casting in 3D printed mold and subsequently dissolving soluble mold material (in organic solvent or water). It results in obtaining a scaffold with a desired shape, being a negative of sacrified mold. The method of provides opportunity to obtain a wide variety of scaffolds without rheology requirement necessary to print by direct extrusion method [2].

Materials and Methods

Indirectly 3D printed hydrogel scaffolds where obtained from 0.5% w/v gellan gum (GG) and 2.5% w/v gelatin (Gel) mixture (both from Sigma Aldrich).
3D models of gyroid shape were algorithmically generated by slicing program (Cura 3, Ultimaker). Gyroid scaffold models were compared with classical lattice models in terms of stress distribution using finite elements method (FEM) in Fusion 360 software (Autodesk).
Gyroid model of the mold (FIG. 1A) was 3D printed from poly(vinyl alcohol) (PVA, Formfutura, Nederland) (FIG. 1B) and polylactide (PLA, Noctua ULTRA-PLA, Poland, as a reference) filament on He3D FDM 3D printer. GG/Gel mixture was molded into PVA (FIG. 1C). PVA was dissolved in water by incubation for 24 h to obtain GG/Gel hydrogel scaffold (FIG. 1D).
Quality of the prints for different infill percentage was examined by optical means (Keyence microscope). Obtained molds were used to produce GG/Gel hydrogel scaffolds. Additionally PVA molds were tested in contact with water to determine maximal gelation timeframe in which mold details are still preserved and can be imprinted into molded hydrogel.

Results and Discussion

It was shown that for the same load gyroid structure was more mechanically stable with lower maximal stress values compared to lattice structure. PVA material was more demanding to print than PLA therefore 4-fold reduction of printing speed was required. Dried PVA showed inferior layer adhesion compared to wet PVA.
It was also shown that any hydrogel with gelation time lower than 2 minutes can be molded in water soluble PVA with minimal detail lost.

Conclusions

The study showed possibility to use indirect 3D printing in PVA molds for fast gelling GG/Gel hydrogels although PVA printing process was more challenging than classical PLA printing.

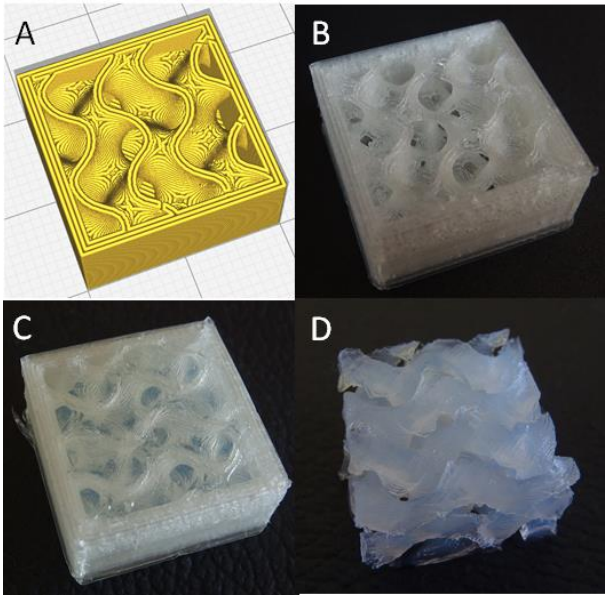

FIG. 1. Gyroid mold model in Cura 3 (A), PVA mold (B), PVA mold after GG/Gel infiltration (C), hydrogel scaffold after PVA removal (D).

Acknowledgments

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COMPARISON OF CERAM X COMPOSITE AND EQUIA FIL FORTE GLASS HYBRID RESTORATIVE SYSTEM ON THE *S. MUTANS* AND *A. VISCOSUS* MONOSPECIES CARIOGENIC BIOFILM FORMATION IN *IN VITRO* MODELS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 94]

Introduction
The main reason mentioned for the chosen of dental materials is the dental biofilm recurrence. In vitro monospecies biofilm models with *Streptococcus mutans* and *Actinomyces viscosus* around dental filling materials like nano-ceramic composite resin Ceram. X (One Universal, Dentsply, DeTrey, Konstanz, Germany) and Equia Forte Fil (GC, Tokyo, Japan) glass hybrid restorative system have been used to this study.
The aim of the present study was to evaluate the antimicrobial efficacy of a glass hybrid restorative compared with a nano-ceramic composite resin on *S. mutans* and *A viscosus* monospecies biofilm models.

Material and methods
In this *in vitro* experimental model, 40 nano-ceramic composites resin (Ceram. X) samples and glass hybrid restorative system (Equia Forte Fil) applied to polystyrene tiles according to the manufacturer's instructions activated by UV light, respectively. Surface roughness was measured using a profilometer and scanning electron microscopy.
S. mutans and *Actinomyces viscosus* monospecies biofilm formation cultured in brain-heart infusion broth suplemented with 5% xylitol, D-sorbitol and sucrose was used for the assessment of biofilm formation and biomass on the samples.
The relationship between the type of restorative materials, cariogenic monospecies biofilm formation and selected substrates was studied.

Results
A statistically significant reduction was found in the mono-species biofilm formation of *S. mutans* and *A. viscosus* under the influence of the application of Equia Fil Forte glass ionomer compared to Ceram X composite *in vitro* models (Fisher's exact, Mann-Whitney U, tests; $P < 0.05$). In addition, a smaller biofilm was observed under the influence of substrates, i.e. 5% xylitol and D-sorbitol compared to sucrose. Xylitol showed a stronger inhibitory effect on the S. mutans biofilm formation on the Ceram X composite compared to D-sorbitol. However, in the case of A. viscosus biofilm, D-sorbitol turned out to be a stronger inhibitor than xylitol.

Conclusions
Cariogenic monospecies biofilm generation have been shown to be related to the type of dental restorative materials and substrates within in vitro models, but these results do not correspond exactly with those obtained from in vivo studies using restorations in dental appliances. Though not conclusive, some in vitro restorative materials like Ceram X have shown that certain materials possessing antimicrobial potential may reduce the severity of lesion formation depending on the type of the substrate used.
This studies suggesting possible pathways for modification in the use of composite like Ceram X and their antimicrobial potential with potentially enhanced longevity and caries prophylaxis.

THE INFLUENCE OF PHASE CHANGE MATERIAL (PCM) ON SELECTED PROPERTIES OF POLYURETHANE/MAGNETITE SYSTEMS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 95]

Introduction

Polyurethanes (PUs) can be defined as the group of polymers which contain urethane linkages [1]. Depending on chemical composition, polyurethanes can exhibit a variety of properties, which can be adjusted to the specific purposes. Therefore these materials are widely investigated nowadays. Due to their good biocompatibility, PUs are considered to be good materials for biomedical applications [2].

Typically polyurethanes are synthesized using three compounds: long-chain polyol, isocyanates and chain extender [2]. Either one- or two-step polymerization can be employed according to the desired properties of the obtained polyurethane. The latter allows better control of the final composition of the product. During the first step, a prepolymer is formed and then it reacts with a chain extender during the second step as shown in FIG. 1.

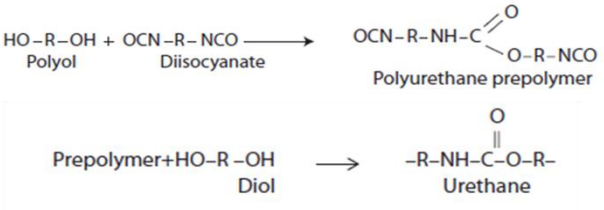

FIG. 3. Reactions during a two-step polymerization of polyurethanes [1].

Phase change materials (PCMs) can store thermal energy during phase transition, due to their high enthalpy of fusion. One of the representatives of PCMs is poly(ethylene glycol), a semi-crystalline polymer which exhibits high degree of crystallinity. Melting temperature and heat of fusion change according to the molecular weight of PEG. Incorporation of PEG PCM material into polyurethane matrix can be utilized to store latent heat of polymerization of PU. This is important in applications as injectable bone cement, where very high temperatures during polymerization can cause necrosis of surrounding tissues [3].

Materials and Methods

Poly(ethylene glycol) (PEG) with M_w = 2000 g/mol *(Sigma Aldrich)*, 1,6-hexamethylene diisocyanate (HDI) *(Fluka Analytical)*, dibutyltin dilaurate (DBTDL) *(Aldrich Chemistry)* as a catalyst, 1,4-butanediol (BDO) *(Sigma Aldrich)* as a chain-extender and sodium alginate (SA) *(Sigma Aldrich)* as a crosslinker have been used in polyurethane preparation. As additives micro magnetite particles (Fe_3O_4) *(Aldrich Chemistry)* and poly(ethylene glycol) (PEG) with different concentration and molecular weight *(Sigma Aldrich)* were used.

One day before the synthesis PEG 2000 was dried at 90°C under vacuum conditions for 2 h. About 2 h before the synthesis, it was placed into an oven at 60°C in order to melt the polymer. Magnetite was dried at 110°C for 12 h. Melted polyol was placed into a three-neck flask to which nitrogen inlet, the cooler and the mechanical stirrer were connected. Temperature was set and kept around 50°C using the heater and the catalyst was dropped. Next, HDI was added. During the reaction, temperature was kept below 60°C for 40 minutes.

Meanwhile, other components: BDO, SA, magnetite and PEG were mixed together in order to obtain a paste.

After 40 minutes, the prepolymer was put into a polypropylene container and the paste was added. The components were mixed and placed into an oven set at 80°C for 2 h. Afterwards, the temperature was decreased to 60°C and the obtained products were kept at these conditions for 12 h.

Microstructure, porosity and in vitro chemical stability in PBS and Ringer solution as well as bioactivity in SBF solution by Kokubo method have been investigated.

Results and Discussion

All the samples exhibit porous structure which was shown in FIG. 2.

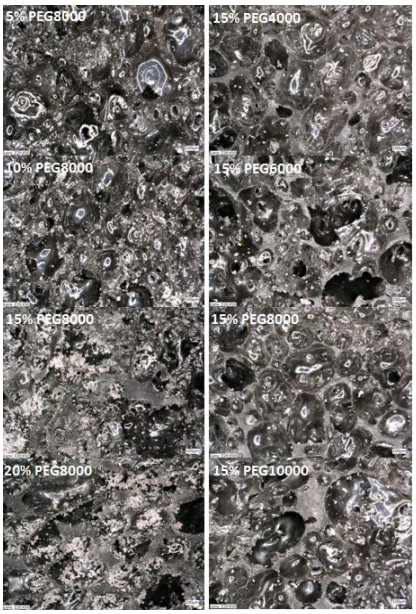

FIG. 2. Pictures of manufactured samples made using digital microscope at 50x magnification.

Porosity of the samples depends on concentration and type of added phase-change material. For the lowest concentration (5%) sample was highly porous.

Conclusions

Polyurethane-based bone scaffolds modified with phase change material (PCM) with different concentration and different molecular weight are multifunctional materials with controlled porosity that can be applied for bone regeneration.

Acknowledgments

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ELECTRICAL AND BIOLOGICAL PERFORMANCE OF OXYGEN AND NITROGEN MODIFIED CNTs

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[ENGINEERING OF BIOMATERIALS 153 (2019) 96]

Introduction

Functionalization of carbon nanotubes is the process that significantly improves their dispersability [1,2] and biocompatibility [3,4]. As some studies suggest, high level of oxidation yields materials that can be easily degraded in the human body [5,6].

For the individual CNTs, the process of covalent modification is known to deteriorate the electrical [7] and mechanical properties, by introducing structural defects. Still, the positive aspect here is the ability to conjugate the materials with various molecules to obtain substrates for drug delivery systems [8]. Meanwhile, the real potential of CNTs functionalization lies within their application as films and layers on the substrate of choice or as a composite fillers. While as films/layers, functionalization improves the CNTs/CNTs and CNTs/substrate adhesion and also act as hole dopant, enhancing electrical conductivity [9,10]. When used as modificators, functionalization enhances CNTs' dispersion within the matrix, resulting in improved mechanical and electrical properties [11]. It is also important to know, that cells are sensitive to surface chemistry of the materials they come in contact with, and different cell types favour [12-15] different conditions.

This study had three objectives: 1) to fabricate oxygen and nitrogen modified CNTs that are easily dispersible and can be used to prepare composite materials; 2) to fabricate and evaluate the physicochemical properties of the composite material, modified with CNTs with different functional groups; 3) to test if the obtained materials are biocompatible and if the fibroblasts prefer any particular morphology and/or functional groups. The tested materials' morphologies were layers and fibres, which are regarded promising in the field of tissue engineering applications.

Materials and Methods

Oxidized CNTs were fabricated according to the procedure established in our previous studies [16,17]. DCC (used for induced amide coupling) and ammonia were used to fabricate the nitrogen modified CNTs.

From these, composite materials were fabricated via: 1) electrophoretic deposition to obtain well-adhered layers on the surface of biocompatible titanium plate and 2) electrospinning of PAN precursor, followed by carbonization, to obtain nanofibrous scaffolds with CNTs embedded inside the carbon nanofibers.

Physicochemical and electrical properties of the CNTs and their composites were evaluated via XPS, FTIR, SEM, 4-point probe measurement. Biological response was studied on L929 fibroblasts. PrestoBlue and Toxilight were used (according to manufacturer's instructions) to assess the materials' biocompatibility, while staining with acridine orange enabled to visualize their morphology.

Results and Discussion

The applied methods of chemical functionalization enabled to obtain easily dispersible CNTs with high amounts of either oxygen or nitrogen atoms. These were used to fabricate composite materials of high quality: well adhered layers and carbon nanofibers with CNTs evenly distributed within the fibres, as manifested by reduced sheet resistivity.

All of the obtained materials were found to be highly biocompatible. During their initial growth, fibroblasts preferred smoother surface of CNTs layers over the fibrous scaffolds, manifested by higher initial adhesion, lower cytotoxicity and higher viability of cells at day 3 of culture. In this initial stage of growth, oxygen functional groups were preferred over nitrogen for both types of materials. At 7th day of culture, cells grown of fibres proliferated extensively, resulting in increased viability and reduced cytotoxicity. Still, the results were better for the layers. At day 7, fibroblasts were found to prefer nitrogen-modified CNTs, regardless of the morphology. The origin of such phenomena is yet to be defined.

Conclusions

Covalent modification is an elegant way to obtain functionalized CNTs with high applicability potential in various fields of materials science, including biomedical. Highly conductive, biocompatible materials can be obtained and cellular reaction of desired cell type can be tailored to meet specific needs.

Acknowledgments

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PROPERTIES OF POLYOXYMETHYLENE COMPOSITES WITH FUNCTIONALIZED HYDROXYAPATITE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 97]

Introduction

Polyoxymethylene (POM) belongs to the class of the thermoplastic polymers showing an excellent endurance, good mechanical properties and wear resistance [1]. However, low thermal stability and resistance to UV radiation limits POM range of applications. From the biomedical applications' point of view it is important to improve POM resistance to higher temperatures, due to the sterilization process. Some bioactive additives introduced to POM catalyse its decomposition during melt processing under elevated temperatures [2]. In the previous studies it has been found that addition of functionalized hydroxyapatite leads to increase of POM thermal stability [3]. In the presented study hydroxyapatite with controlled nanoparticles size and shape was functionalized with poly(ethylene glycol) (PEG) of average molar mass 2000, and the influence of HAp-g-PEG on POM composite properties was investigated.

Materials and Methods

Hydroxyapatite with needle like shape and particle size of 60 nm were used. Functionalized hydroxyapatite was obtained by grafting of poly(ethylene glycol) using 1,6-hexamethylene diisocyanate as a coupling agent. The obtained HAp-g-PEG was introduced into the polyoxymethylene matrix using the extrusion methods and shaped by injection moulding.

Obtained POM/HAp-g-PEG composites were tested using infrared spectroscopy (FTIR), thermogravimetry (TG) and differential scanning calorimetric (DSC). The degree of crystallinity and crystallization rate were calculated on the basis of DSC curves obtained under isothermal conditions.

Results and Discussion

The effects of PEG grafting on hydroxyapatite were confirmed by FTIR results. The absorption bands at 3200-3450 cm^{-1} confirmed formation of urethane groups (FIG. 1). A decrease in the intensity of absorption band at 3571 cm^{-1} , arising from hydroxyl group in HAp, and the presence of new bands in the characteristic ranges for urethane groups, confirm the successful PEG grafting on the surface of HAp. Thermogravimetry analysis shows that obtained HAp-g-PEG contained 30% of organic phase. From TG data of POM/HAp-g-PEG composites, it can be seen that the addition of HAp-g-PEG increased the thermal stability of the composites from 274ºC for pure POM to 327ºC for POM with 0.5% of HAp-g-PEG.

The analysis of DSC curves did not show any significant differences between unmodified polymer and composite with HAp-g-PEG.

An endothermal peak of POM melting was observed at 165°C. Melting point and crystallization temperature of nanocomposites were determined from DSC curves. The degree of crystallinity was calculated based on DSC results. For unmodified polymer, degree of crystallinity was ca. 49% and with the increase HAp-g-PEG concentration, degree of crystallinity slightly decreased to 44% for POM with 5% of HAp-g-PEG.

From isothermal DSC results the relative crystallinity was calculated (FIG. 2) and next Avrami coefficient and rate of crystallization were determined (FIG. 3). It was observed that the time of crystallization increases with the increase of crystallization temperature.

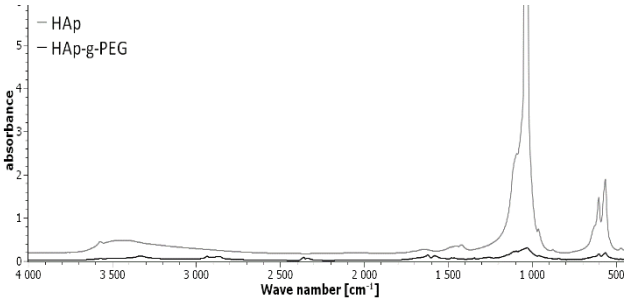

FIG. 1. FT-IR spectrum of HAp and HAp-g-PEG.

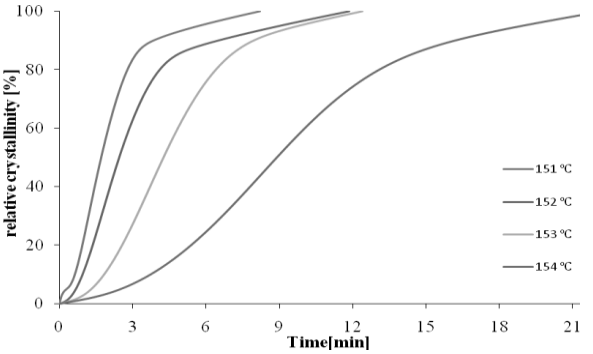

FIG. 2. Relative crystallinity as a function of crystallization time of nanocomposite POM/5%HAp-g-PEG.

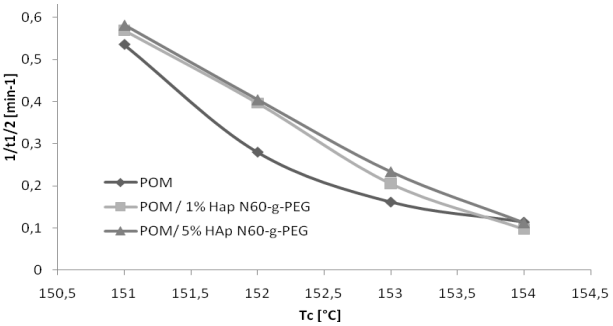

FIG. 3. Total crystallization rate as a function of crystallization temperature for POM and nanocomposites POM/HApN60-g-PEG.

Conclusions

Based on the presented results it can be concluded that the addition of HAp-g-PEG significantly improves the thermal stability of polyoxymethylene (increase of 50°C for 0.5% of the additive concentration). No significant effect of the additive on the properties such as rate and degree of crystallization, crystallization temperature and melting point was observed.

Acknowledgements

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INTRODUCTION OF GRAPHENE OXIDE, REDUCED GRAPHENE OXIDE AND HYDROXYAPATITE INTO CHITOSAN HYDROGEL MATRIX – CHANGES IN MECHANICAL BEHAVIOUR

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[ENGINEERING OF BIOMATERIALS 153 (2019) 98]

Introduction

Since 1960, when they were firstly used and described as a material for contact lenses production, hydrogels have been continuously developed. Recent years shown that the interest in these materials has increased, especially in medical, dental and pharmaceutical applications e.g. drug carriers, wound treatments, capsules matrices and coatings. Modification of hydrogel allow to change and control various properties e.g. level of active compound release, water absorption/dehydration rate, electrical and magnetic response, etc.

One of the most interesting and promising modifiers that can be introduced into hydrogel matrix is graphene and graphene oxide. Considerable progress in reducing costs of production increased accessibility of these materials. Due to their low density, unique electrical, thermal and optical properties, they seem to be an excellent modifying phase in composites. Moreover, high solubility of graphene oxide in water imparts its feasibility as new filler for reinforcement hydrophilic biopolymers.

Chitosan is a natural polymer that has been studied extensively over several decades. It possesses a number of interesting properties including biocompatibility, biodegradability, and affinity to water. It is used in separation membranes, artificial skin, bone substitutes, tissue engineering, coatings and water treatment. Unfortunately its mechanical properties are rather poor thus a wider range of application, especially in a medical field is limited. By combining the advantages of graphene oxide, chitosan and naturally present in bones inorganic phase eg. hydroxyapatite, different types of promising materials for medical applications can be obtained.

Materials and Methods

In this study a natural polymer matrix – hydrogel based chitosan (CS) – was reinforced with three types of particles: graphene oxide (GO) (ITME, Poland), reduced graphene oxide (rGO) (ITME, Poland) and hydroxyapatite (HAp) (mkNano, Canada). Several variations of composite foils were obtained by solution-evaporation casting method with certain percentage of compositions. Into hydrogel based chitosan matrix following modifiers were introduced: CS/GO, CS/rGO, CS/HAp, CS/GO/HAp, CS/rGO/HAp. To examine their mechanical properties static uniaxial tensile tests and analyses of strain-stress curve were performed (Zwick 1435). Tensile strength R_m, Young's Modulus E and Maximal deformation ε_{Fmax} were characterised. Due to the nature and geometry of samples tests were difficult to conduct. Thin hydrogel based composite foils are very sensitive to an air humidity. When they were drying out they became to shrink and to coil up.

Results and Discussion

Based on the results it can be concluded that the introduction of the graphene family nanofillers into hydrogel based polymer matrices effects on their mechanical parameters. The largest strengthening effect was obtained by adding a nanofiller to the hydrogel matrix in the form of reduced graphene oxide.

The addition of the ceramic microphase (HAp) to the chitozan/graphene and chitozan/grapheme-oxide matrix significantly decreased the Young's modulus and the tensile strength. It is supposed that HAp particles impede structural integrity thus mechanical parameters of the composite drop.

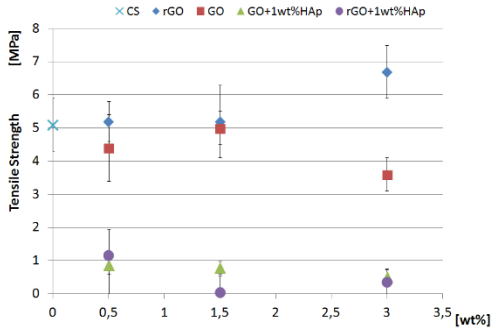

FIG. 1. Tensile strength of different hydrogel composites.

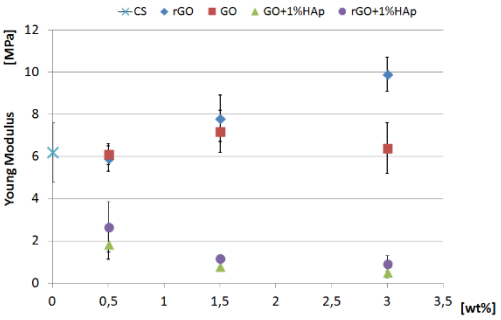

FIG. 2. Young modulus different hydrogel composites.

Conclusions

The hydrogel/graphene oxide and hydrogel/graphene oxide/hydroxyapatite composites were successfully obtained by using solution-evaporation casting method. Addition of different content of nano- and microfillers allows to control and modify mechanical properties of the composites. This approach can be exploit for fabrication of a new, multifunctional material for biomedical applications. Tailored properties of such implant should improve bone or cartilage tissue regeneration.

Acknowledgments

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DEVELOPMENT OF FUNCTIONAL HYDROGELS FOR CONTROLLED ION RELEASE

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[ENGINEERING OF BIOMATERIALS 153 (2019) 99]

Introduction

Tissue engineering seeks to mimic the natural extracellular matrix (ECM) using materials and bioactive factors to control cellular responses. The typical components used in tissue engineering are cells, biomaterials and biomolecules/bioactive factors [1].
Recently, hydrogels have gained popularity among biomaterials used as 3D scaffolds for tissue regeneration. The combination of their unique properties like biocompatibility, high water content, permeability, hydrophilicity, physical properties and chemical structure made them ideal candidates for different biomedical applications in tissue regeneration [2].
There is a broad variety of biomolecules/bioactive factors employed for Tissue Engineering applications such as growth factors, hormones, peptides, components of the extracellular matrix, vitamins or ions, all of them possibly promoting cell proliferation and/or differentiation [1].
Boron is a trace microelement essential in the metabolism of living organisms. Yet, its exact role in mammalian cells and the precise mode of action at the molecular level had not been well defined. Recent scientific achievements have described a novel activity of boron related to myogenic differentiation [3] and vascularisation [4]. This work aims to optimise the engineering of hydrogel-based material systems capable of sustained boron-release for Tissue Engineering applications.

Materials and Methods

Two different types of hydrogels have been tested. The hydrogel-based material were composed of alginate and polyethylenglycol (PEG), and they were loaded with two different concentrations of boron 0.59 mM (Boron low) and 1.47 mM (Boron high). Additionally, three types of PEG hydrogels were designed: PEG 3% (30 mg/ml), PEG 5% (50 mg/ml) and PEG 10% (100 mg/ml).
The long-term release assay was conducted to assess the boron delivery from hydrogel systems. The aim of the experimental design was to analyze the relation between the hydrogel composition and the boron-delivery. Additionally, hydrogel characterisation and optimisation of hydrogel production. Boron liberated from hydrogels was assessed by colorimetric techniques, measuring absorbance using azomethine reaction that occurs specifically with boron. Mechanical (TMA) and thermogravimetric analysis (TGA) was used to investigate the effects of boron on the mechanical and thermal properties of the hydrogels.

Results and Discussion

The boron concentration measurements, TGA and TMA studies confirm that boron interacted with both hydrogels (alginate and PEG). As the quantity of boron inside the PEG hydrogel polymer chain is quite high. It creates a possibility to release the ions during sample degradation. Boron remains cross-linked in the PEG polymer chain independently from the concentration of PEG and its cross-linker. The majority of boron ions are liberated after one day of immersion. Boron-loaded alginate hydrogels release similar boron concentrations. However, TGA results indicated that with the increase in the amount of boron, the percentage of residual mass increases. Taking into consideration all performed tests it can be suspected that boron is covalently cross-linked with polymer chains.

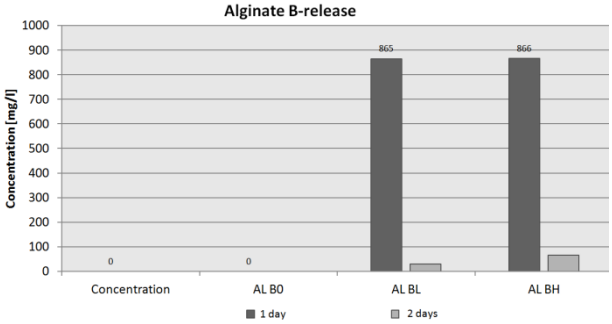

FIG. 1. Boron-release from alginate hydrogels.

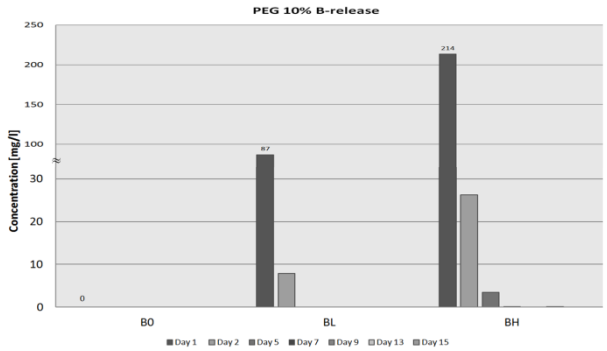

FIG. 2. Boron-release from PEG 10% hydrogels.

Conclusions

Controlled boron ions release hydrogels could be a promising tool for future *in vivo* applications. Even after one month they could be applied by an injection. This work should be treated as the first step of development and optimisation of functionalised hydrogels.

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DESIGN AND MANUFACTURE OF CUSTOMIZED MEDICAL IMPLANTS - CONTINUED

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[ENGINEERING OF BIOMATERIALS 153 (2019) 100]

Introduction

Last year, we reported the commencement of work under the POIR programme 1/4.1.4/2017 financed by the Polish National Center for Research and Development. The project concerns design and manufacture of customized, osseointegrated percutaneous orthopaedic implants, intended for people after amputation of the leg above the knee. Such an implant allows the load to be transferred directly from the femur to the prosthesis omitting soft tissues usually involved in this process when using a socket-suspension type prosthesis system. In the area of design work, our project is currently advanced in about 70%.

Materials and Methods

Implants were design using reverse engineering, biomodelling, and CAD software: Geomagic Design X (3D Systems, USA) industry standard reverse engineering software with advanced mesh editing tools; Solidworks (Dessault Systems Solidworks Corporation, USA) one of the leading software applications used for mechanical design; Geomagic Freeform (3D Systems, USA) a voxel based biomodelling software package that converts models into virtual clay that can be manipulated much like physical clay. All finite element analyses were performed using ANSYS R19.1 (ANSYS, Inc. Canonsburg PA, USA). The project of prototype implant was analyzed from a manufacturing perspective using CAM software (Hypermill, OPEN MIND Technologies AG, Germany) and CNC milling was performed with the use of hybrid milling system (Laser 1300, C.B. Ferrari, Italy). Implant prototypes were sterilized with hot dry air. Thrombo-compatibility was assessed using scanning electron microscopy and flow cytometry tests, cytotoxicity was assessed by XTT test, and genotoxicity was assessed by micronucleus test.

Results and Discussion

Using commercially available engineering programs, we made 19 subsequent versions of the implant prototype fitted to the anatomical structures of the patient's amputated bone. Subsequent versions took into account both orthopaedic remarks and the results of numerical analysis by the finite element method. The last version of the prototype, made in a series of 10 copies, after evaluation of microbial pollution and sterilization, is currently undergoing strength and fatigue tests.
At the same time, tests of thrombo-compatibility as well as cytotoxicity and genotoxicity of materials planned for use in the manufacture of the final medical devices are carried out, taking into account their surface modification made in order to achieve effective integration with bone tissue and limiting the development of microorganisms in the place of contact and penetration of soft tissues.

Conclusions

To date, we have not encountered insurmountable difficulties and we are approaching the moment when we prepare implants for patients currently selected for clinical procedures.

Acknowledgments

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OSTEOCONDUCTIVE POTENTIAL OF PLGA/BIOGLASS COMPOSITE BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 153 (2019) 101]

Introduction

Composite materials with the polymer matrix and bioactive glasses as modifiers are potential materials for regenerative medicine [1]. It is known, however, that in addition to the chemical composition, important factors determining the bioactivity of the biomaterial are also its surface properties and structure [2].
Compared to two-dimensional materials, porous three-dimensional materials, due to having interconnected pores of the right size, facilitate cell adhesion, proliferation, differentiation and even tissue regeneration in a more natural way, because their structure resembles living tissue [3].
The aim of this study was to investigate the effect of two- and three-dimensional PLGA/bioglass composite biomaterials on *in vitro* cell culture, ultimately on normal human osteoblasts (NHOst). The reason for these considerations was potential application of the biomaterials in tissue engineering for regeneration of bone tissue losses.

Materials and Methods

Materials

PLGA/BG composite materials in the form of foils (2D) and porous scaffolds (3D) were fabricated using solvent casting and solvent casting particulate leaching techniques, respectively. Dichloromethane and NaCl (315-400 μm) were used as a solvent and porogen, respectively. The molar ratio of L-lactide to glycolide in the copolymer was 85:15. The volume fraction of BG in the composites was 21%. Bioactive glass particles (<45 μm) with chemical composition of (mol.%) $80SiO_2$-$16CaO$-$4P_2O_5$ (S2) and $40SiO_2$-$54CaO$-$6P_2O_5$ (A2) were prepared using sol-gel method. The biomaterials thus obtained were tested for biocompatibility and functionality.

Cell study

The effect of biomaterials on bone cell differentiation was studied by testing the activity of early and late markers of bone formation: alkaline phosphatase (ALP), osteopontin (OP) and osteonectin (ON). Normal human osteoblasts (Lonza, USA) were cultured with biomaterials for 7, 14, and 21 days. The activity of ALP was measured after 7 and 14 days of cell culture using 4-MUP (Sigma, USA) test. The level of OP and ON secretion was measured with ELISA tests (Cloud-Clone Corp., USA).

Results and Discussion

The effects of conducted studies suggest a positive influence of bioglass additives and 3D structure on cell differentiation. Among the studied biomaterials the best results showed the 3-dimensional composite material containing A2 bioglass (FIG. 1).

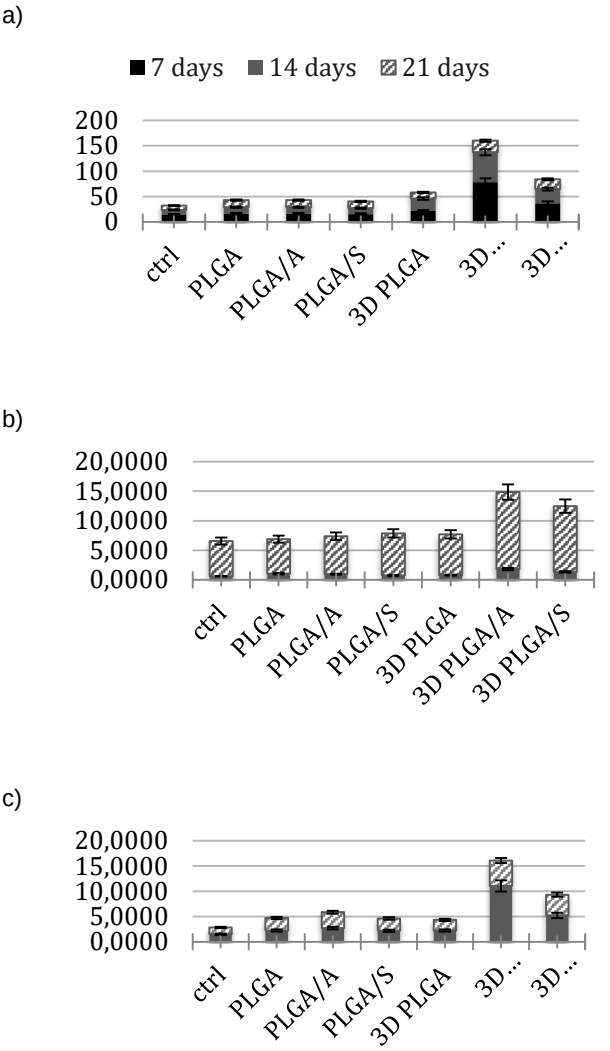

FIG. 1. a) The activity of ALP, and the level of b) ON and c) OP secreted by NHOst cultured on biomaterials.

Conclusions

The addition of bioglass together with the 3D structure of composite significantly increases the osteoconductive potential of obtained biomaterials.

Acknowledgments

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HYDROXYAPATITE/CHITOSAN HYBRID-BASED BIOMICROCONCRETES AS NOVEL BONE SUBSTITUTES – IN VITRO STUDIES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 102]

Introduction

Hybrid materials gained great attention in recent years. In our study materials composed of hybrid hydroxyapatite/chitosan (HAp/CTS) granules as aggregate, αTCP as a setting phase, and pectin solutions as a liquid phase, were developed. Hybrid granules combine advantages of hydroxyapatite (i.a. biocompatibility, bioactivity) and CTS (i.a. rapid biodegradation, high biocompatibility and antibacterial properties).

Materials and Methods

The following components were used to prepare biomicroconcretes: hydroxyapatite/chitosan (HAp/CTS) hybrid in the form of precipitate (18% dry weight), fine powder and granules (300-400um), as well as α-TCP powder, and pectin solutions. HAp/CTS hybrid materials, containing different chitosan content (15, 20, and 25 wt.%), and highly reactive α-TCP powder were synthesized using a wet chemical method. Two types of low esterified amidated pectins from citrus peels (CP) and apple pomace (AP) were used. The materials were prepared by mixing the HAp/CTS phase, α-TCP powder and pectin solutions. Materials were incubated in simulated body fluid (SBF). Mass change, swelling, phase composition (XRD, FTIR) and microstructure (SEM/EDX) of the biomicroconcretes were monitored during incubation. Furthermore, the changes in pH, as well as calcium and phosphate ion concentration (ICP-OES) in SBF were measured.

Results and Discussion

The biomicroconcretes showed a gradual increase in mass during incubation in SBF (FIG. 1A). Mass changes can be related to two processes: degradation of the materials (especially organic phase – chitosan and pectins) and inorganic phase changes (α-TCP transformation to HAp and HAp precipitation as a result of SBF supersaturation). The results indicated that the second process is predominant. However, after 35 days of incubation, biomicroconcretes containing HAp/CTS hybrid phase with the lowest content of CTS (15 wt.%) exhibited the highest increase in mass. It may indicate that after a longer incubation period degradation rate of the materials composed of HAp/CTS hybrid phase with a higher content of CTS (20 and 25 wt.%) was higher. In the first day of incubation, the swelling ratio depended on CTS concentration in hybrid phase – the highest value was recorded for HAp/25CTS_AP material (FIG. 1B). Between 3rd and 14th day of incubation, the swelling ratios of all materials reached similar values (~10%) and did not change significantly. After longer incubation periods (21 and 35 days), the values increased with increasing CTS concentration in hybrid phase.

Phase composition analysis showed that just after 3 days of incubation in SBF almost complete transformation of α-TCP to HAp occurred (TABLE 1). Furthermore, the gradual decrease of calcium and phosphate ion concentration in SBF, as well as SEM observations indicated the formation of HAp layer on the surface of biomicroconcretes, indicating their bioactivity.

TABLE 1. Phase composition of the biomicroconcretes based on XRD analysis.

Sample name	7 days in air		3 days in SBF		7 days in SBF	
	αTCP [wt%]	HAp [wt%]	αTCP [wt%]	HAp [wt%]	αTCP [wt%]	HAp [wt%]
HAp/15CTS_AP	25	75	3	97	1	99
HAp/20CTS_AP	22	78	3	97	2	98
HAp/25CTS_AP	23	77	4	96	1	99

Conclusions

Obtained biomicroconcretes showed bioactivity and rapid transformation of setting phase (α-TCP) to HAp. The results indicated that the degradation and swelling rates of the materials can be modulated by the use of HAp/CTS hybrid phase with different chitosan concentration.

Acknowledgments

This work was supported by the National Science Centre, Poland Grant No. 2017/27/B/ST8/01173.

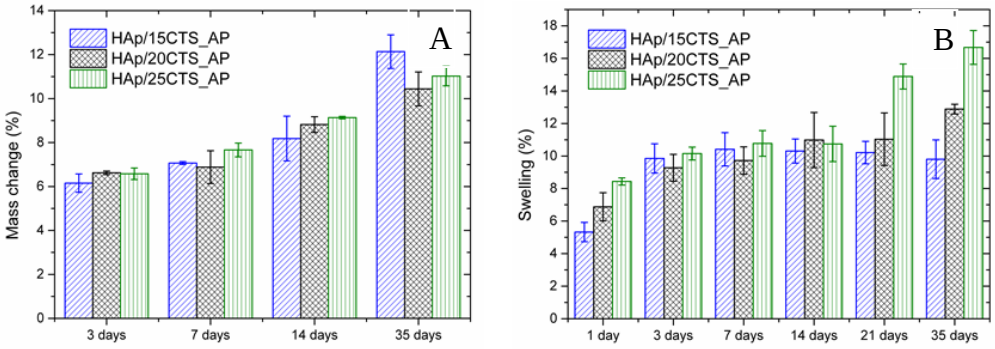

Figure 1. Mass change and swelling of the biomicroconcretes during incubation in SBF.

INFLUENCE OF CHITOSAN NONWOVEN FABRIC MODIFICATION ON ITS PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 153 (2019) 103]

Introduction

The surface modification of the non-woven fabric, which is an architecturally close substrate for the external cellular matrix (ECM), is an attractive way to obtain scaffolding that stimulates the regenerative processes of the damaged tissue. Studies on scaffolds functionalization focus on the attachment of proteins or peptides activating adhesion processes [1]. Unfortunately, proteins tend to randomly curl during adsorption, making binding domains inaccessible to their cell membrane counterparts. In addition, since native ECM proteins may have several binding motifs, there is some difficulty in predicting the cellular response when used. Therefore, only protein fragments - short peptides containing specific motifs recognized by receptors - are used [2]. Such a solution guarantees greater stability of the system in relation to heat treatment, sterilization, pH changes or in vivo environment [3]. In the literature, examples of peptides derived from fibronectin (e.g. RGD, REDV, KQAGDV), collagen (e.g. DGEA, GFOGER), laminin (LRE, PDGSR, IKVAV, YIGSR) or elastin (e.g. VAPG) can be found [2,3]. The most commonly used of these is the arginine-glicyine-aspartate (RGD) sequence, which occurs in most ECM matrix proteins and acts as an adhesive [2].
Modification of nonwovens, which additionally have the character of partially cross-linked hydrogel, is challenge. Proceedings of chemical modification may change the attractive microstructure and physical methods of depositing - the results are insufficient for the needs (low concentration of peptides on the fiber surface) [4]. Additionally, the processing of the finished material significantly influences its physical properties (fibre morphology). The electrospraying method proposed in the paper seems to be a technique bringing significant advantages in comparison to the classical dip coating. Peptides being an extinguished part of biologically active collagen were applied by means of an electrodischarge device. Modified nonwovens were compared to the behavior of control materials (free of peptides).

Materials and Methods

Collagen-like peptides - K1 and K2 - were synthesized in the Institute of Organic Chemistry of the Technical University of Łódź. Modification of fibre surface was carried out by dip coating and lyophilization (method 1) and electrospraying and freeze drying (method 2).
In Method 1, the first step was to immerse the CS nonwoven fabric in 0.5% peptide solution (PBS/24h/12ºC), the next step was freeze drying of fibres with peptide (-50ºC/0.03Torr/24h). In Method 2, the first step was to natrsy the nonwoven fabric (CS) with pepethide dissolved in water (conditions: 0.25-0.1%/15kV/15min) followed by 24-hour lyophilization (-50ºC/0.03Torr). The obtained nonwovens were examined for morphology: NOVA NANO SEM 200 observing the changes on their surface and the distribution of the fiber size. Tests of absorbability (%N) of nonwovens before and after modification were carried out. The presence of biomolecules in fibrous scaffolds were confirmed by FTIR. The biological test: biocompatibility of the fibrous scaffolds (cytotoxicity, viability, adhesion to the scaffold) in contact with the fibroblastes were tested.

Results and Discussion

Physical modification of nonwovens with peptide solutions leads to a change in the morphology of the substrate, in the case of method 1 the visible peptide grid significantly reduces the copper distances, in the case of method 2 the peptides are adsorbed to the fiber surface. This is confirmed by spectroscopic studies where characteristic bands typical for peptides (I and II order amides) can be found. Thus, the average fiber thickness increases and the fiber becomes rough. It seems that visible changes may have a real effect on cell response, especially in terms of cell adsorption and adhesion.

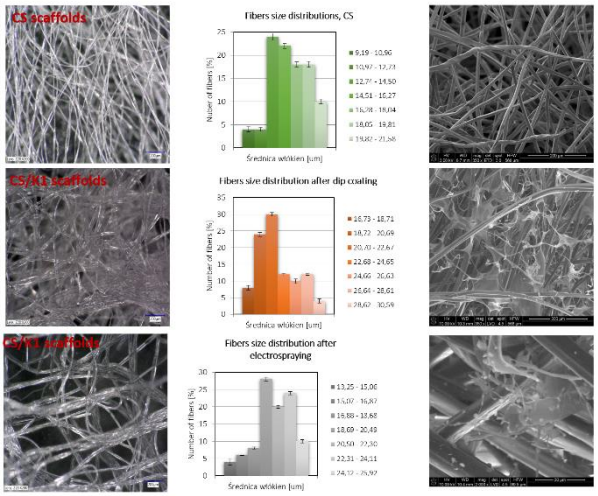

FIG. 1. Microstrucuture of chitozan non-woven fabric (CS/K1) after modyfication method 1 and method 2 with fibers size distribution.

Conclusions

Both dip coating and electrospraying are methods of fibrous substrate modification. In the case of the method 1, the substrate gains an additional microstructure made up of peptides. In the case of method 2, peptides adsorb to the fibres, affecting their surface development and physicochemical changes of the fibres. Thus, the substrates gain biomimetic character conducive to cell proliferation.

Acknowledgments

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