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31st Biomaterials in Medicine and Veterinary Medicine

Annual Conference

13 – 16 October 2022 Rytro, Poland

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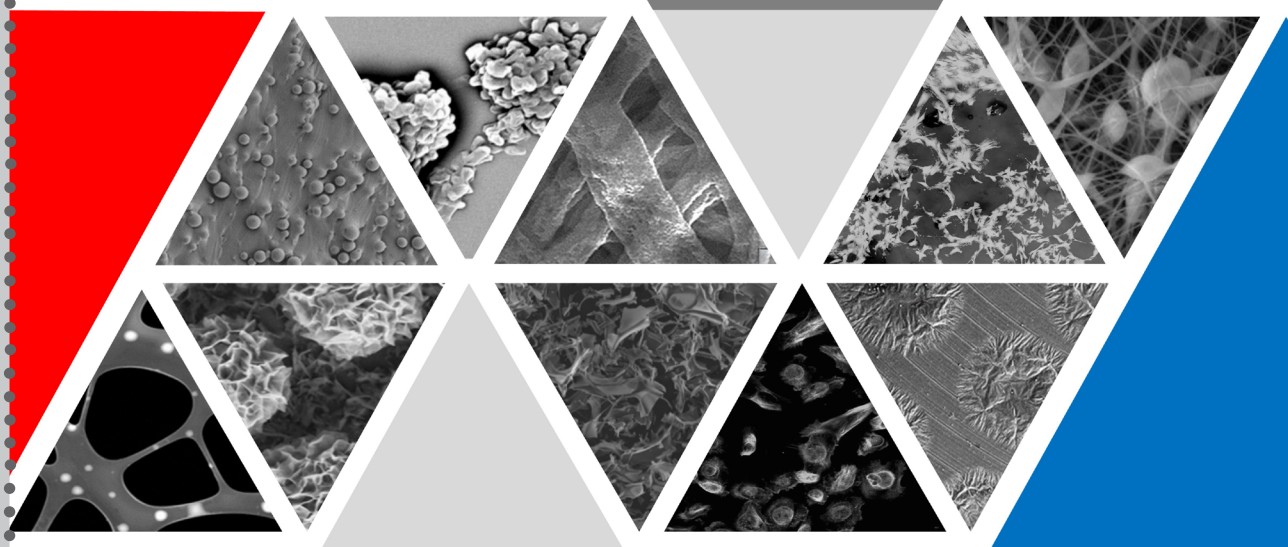

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The Journal Engineering of Biomaterials publishes refereed original articles and review papers on biomedical aspects of engineering. It deals with application of materials engineering principles and methods to problems associated with human health.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

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STUDIA PODYPLOMOWE
Biomateriały – Materiały dla Medycyny
2022/2023

Organizator: Akademia Górniczo-Hutnicza im. Stanisława Staszica w Krakowie Wydział Inżynierii Materiałowej i Ceramiki Katedra Biomateriałów i Kompozytów **Kierownik:** prof. dr hab. inż. Elżbieta Pamuła **Sekretarz:** dr inż. Małgorzata Krok-Borkowicz	**Adres:** 30-059 Kraków, Al. Mickiewicza 30 Pawilon A3, p. 208 lub p. 210 tel. 12 617 44 48, 12 617 23 38 email: epamula@agh.edu.pl; krok@agh.edu.pl https://www.agh.edu.pl/ksztalcenie/oferta-ksztalcenia/studia-podyplomowe-kursy-doksztalcajace-i-szkolenia/biomaterialy-materialy-dla-medycyny/

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Tematyka prezentowana w trakcie zajęć obejmuje przegląd wszystkich grup materiałów dla zastosowań medycznych: metalicznych, ceramicznych, polimerowych, węglowych i kompozytowych. Słuchacze zapoznają się z metodami projektowania i wytwarzania biomateriałów a następnie możliwościami analizy ich właściwości mechanicznych, właściwości fizykochemicznych (laboratoria z metod badań: elektronowa mikroskopia skaningowa, mikroskopia sił atomowych, spektroskopia w podczerwieni, badania energii powierzchniowej i zwilżalności) i właściwości biologicznych (badania: *in vitro* i *in vivo*). Omawiane są regulacje prawne i aspekty etyczne związane z badaniami na zwierzętach i badaniami klinicznymi (norma EU ISO 10993). Słuchacze zapoznają się z najnowszymi osiągnięciami w zakresie nowoczesnych nośników leków, medycyny regeneracyjnej i inżynierii tkankowej.

Sylwetka absolwenta:
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Zasady naboru:
Termin zgłoszeń: od 20.09.2022 do 20.10.2022 (liczba miejsc ograniczona - decyduje kolejność zgłoszeń)
Wymagane dokumenty: dyplom ukończenia szkoły wyższej
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POLYELECTROLYTE AND PROTEIN ASSEMBLIES FOR THE DESIGN OF FUNCTIONAL THIN FILMS AND MATERIALS

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Abstract

Layer-by-layer (LbL) assembly is a surface modification technique based on the alternate deposition of polycations and polyanions. Incorporating proteins in LbL thin films presents a major interest for biotechnologies and biomedical sciences. It can indeed allow enzymes to be immobilized in bioreactors, cell signaling cues to be presented at the surface of a biomaterial, or drugs/growth factors to be released from a medical device. The assembly of homopolyelectrolytes mainly rests on electrostatic interactions. After each layer deposition, charge overcompensation occurs, thereby making the interaction with the next layer possible. Proteins are polyampholytes, with a conformation that is much more constrained than the one of homopolyelectrolytes, and with a patchy distribution of charges and of polar/apolar groups that differs from one protein to another. For these reasons, assembling proteins with the LbL technique is challenging, notably because charge overcompensation cannot be achieved. For example, the LbL assembly of collagen and fibronectin, two major adhesion proteins of extracellular matrices, was shown to level off after the deposition of a few layers (FIG. 1a) [1].

Successful assembly was reported for different systems combining proteins and homopolyelectrolytes, which will be reviewed. For applications in biomaterials science and tissue engineering, the use of natural polyelectrolytes is recommended. With a view to create biomimetic environments for cells, collagen and hyaluronic acid were for example assembled using the LbL approach. Moreover, such assembly was performed in templates featuring intersected nanopores. After template dissolution, biomimetic membranes made of intersected nanotubes were obtained (FIG. 1b), which are self-standing if strengthened using mineral particles. Such membranes support cell adhesion, and could be further used to release signaling molecules embedded in the walls or the core of the nanotubes.

Parameters favoring the growth of LbL thin films must be carefully adjusted for each new protein to be assembled, and may even sometimes not be identified. Standardizing the surface properties of protein molecules would be a way to extract more general rules for their assembly. Protein-polyelectrolyte complexes (PPCs) may then become very interesting building blocks for the incorporation of proteins into LbL assemblies. It was demonstrated, in a proof-of-concept experiment, that the growth of LbL films was better for lysozyme complexed with poly(styrene sulfonate) (PSS) compared to single lysozyme molecules (FIG. 1c) [2]. The activity of lysozyme was moreover higher, which is attributed to the higher polyelectrolyte content of the layers built with PPCs. Further results have shown that PPCs are a means to incorporate proteins in LbL assemblies based on more robust and generalized design rules [3].

Applications of such coatings will be illustrated. LL-37, an anti-microbial peptide, was incorporated in thin films designed to reduce infection of orthopaedic prostheses [4]. Glucose oxidase was immobilized in nanopores to study the effect of confinement on its activity [5].

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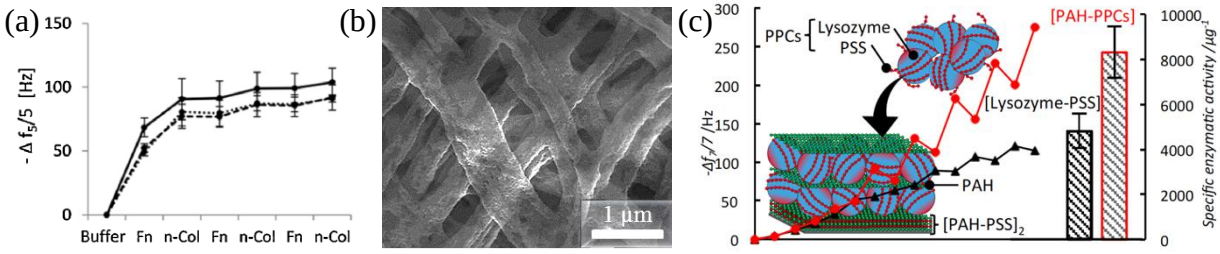

FIG. 1. (a) Cumulated frequency change, recorded by QCM, upon LbL construction with fibronectin (Fn) and collagen (n-Col) (three different buffers), (b) Biomimetic membrane built by LbL assembly of collagen, hyaluronic acid and silica particles in a template featuring intersected nanopores, (c) QCM data (left axis) showing the incorporation of lysozyme-PSS complexes (PPCs) in LbL assemblies with poly(allylamine) (PAH) (red), compared to the assembly of free lysozyme with PSS (black), and the corresponding specific enzymatic activity (right axis).

ADVANCED COATINGS - IMPORTANT FINISH FOR MEDICAL DEVICES AND IMPLANTS

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Introduction

The interfacial area between synthetic biomaterial and cells or living tissue is a front line between two different worlds. Various processes on this boundary may lead to cell death, foreign body response, inflammation, and implant rejection. These processes frequently decide on the success or failure of complex medical procedures and may even have fatal consequences for a patient. Dedicated surface treatment, application of a particular intermediate layer, and special coating can influence various biological processes and can make the device biocompatible. The coating can make the surface hydrophilic or hydrophobic, depending on the application. After coating, the friction coefficient against epithelial or endothelial tissue can decrease by the order of magnitude, or the surface can be mucoadhesive. The coating may prevent bacterial or protein adhesion or can promote the adhesion of dedicated endothelial and muscular cells. The presence of the specific peptides achieves this. The coating may decrease platelet activation, essential in blood-contacting medical devices. Depending on the application, the coating can be made biodegradable or permanent.

Materials and Methods

Several types of surface modification were designed and applied to various biomaterials (polyurethane, PTFE, PLA, PVC, stainless still S316…) and coated using different methods. To prepare a desired coating layer single-step or sometimes multi-step coating processes can be employed. Biomaterials employed in tests were in the form of plane sheets, tubes (catheters), 3D printed structures, and spun nanofibrous sheets and tubes.

- Polyvinyl pyrrolidone (PVP) based covalent and physically attached hydrophilic coatings
- Graphene oxide based coatings on stainless steel
- Poly acrylic acid (PAA) grafting alone and followed by further chemical modification with peptides.
- Dipalmitoyl-phosphatidyl-choline (DPPC), cell membrane mimicking coating
- Oxidative polymerization of various catecholamine alone and followed by further chemical modification with peptides.

Selected coatings were examined by appropriate methods, according to their planned applications. We have estimated the following properties: cytotoxicity, friction coefficient against epithelium, wetting angle, protein adhesion, cell adhesion, platelet activation, and aggregate formation.

Results and Discussion

Obtained materials and coatings were tested in contact with pig's tissue to estimate the influence of the coating type on the friction coefficient. Coated samples were exposed to E. coli and P. mirabilis to determine bacteria adhesion and bacteria ability to travel along coated surface. This phenomenon is responsible for numerous infections during catheterization of urological tracks. Conducted work revealed that coated surfaces were partially resistant to bacteria adhesion and biofilm formation. Also, P. Mirabilis shows a much lower ability to travel across the coated polymer surface. We have tested the coatings in static and dynamic contact with human serum and whole blood. Protein adhesion and various signs of platelet activation were estimated. Experimental work proves that nano-brushes of hydrogel polymer coatings effectively prevent protein adhesion, platelet activation, and platelet clusters formation. Some samples were implanted in rabbits to check long-term interaction with animal tissue.

Further investigation revealed the lack of inflammation and foreign body response in the surrounding tissue and toxic effects on remote organs. Finally, human endothelial cell adhesion to the surface modified with nano-scale (7-12nm) constructs were estimated, showing that some amino acid sequences can promote endothelium adhesion and anchoring to the surface. This technology can produce hybrid implants, lifeless, mechanically robust systems covered by self-healing active coating for the patient's cells. Such a coating is mainly interested in vascular and heart prosthesis since endothelium actively prevents platelet activation by releasing signaling molecules, glucose and oxygen is supplied directly from the bloodstream, and such a coating has self-healing properties. When one cell dies the neighboring one divides and fills the gap.

Conclusions

Physical PVP coating is a chip and versatile method for disposable catheters (urological and vascular), and can be applied to PVC, Pebax and polyurethanes. Such coatings show low friction coefficient and prevents platelet activation and bacteria adhesion. They can also be used as drug releasing coatings. More sophisticated PVP free radical grafting can be used for long term implants. It gives superhydrophylic non adhesive bioinert coatings. Free radical grafting of acrylic acid monomer also gives hydrophilic surfaces which can be covalently modified with peptides for selective or non-selective cell adhesion.

Polycateholamine coatings are simple to apply versatile method to modify – coat almost any type of surface, from Teflon, Silicone to other polymers, glass, ceramics and metals. This type of coating is superhydrophilic due to the presence of surface nanoporosity, biocompatible and offers perfect adhesion sites for cells. Freshly made cateholamine coating reacts with amine groups what allows covalent modification with various compounds including peptides.

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ERC INDIVIDUAL AND SYNERGY GRANTS

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Introduction

The European Research Council (ERC) is a leading European funding body supporting excellent investigator-driven frontier research across all fields of science. ERC calls are open to researchers around the world.

Materials and Methods

The ERC offers various different outstanding funding opportunities with grants budgets of €1.5 to €3.5 million for individual scientists. All nationalities and career-stage of applicants are welcome for projects carried out at a host institution in the European Union and its associated countries.

This presentation will focus on how ERC funds research projects in particular in / around the target topics of the conference. The purpose is to emphasize the career opportunities for the researchers, who work at the frontier of chemical science in areas such as physical, analytical and theoretical chemistry, synthetic and material chemistry as well as chemical and material engineering.

We will remind what the eligibility criteria are, and which call might suit your career stage.

If you have a visionary idea, we will give you some tips to design an individual proposal allowing you to build a team aiming at developing and implementing this idea.

If this idea is so broad that it reaches beyond your own expertise, then we will lead you towards the synergy program in which you have to associate with one to three other investigators whose expertise is key to complement yours and bring the project to success.

Conclusions

At this session, the main features of ERC individual and synergy grants will be presented, as well as testimonials from an ERC grantee in the field of Material Sciences.

Young investigators as well as advanced researchers who work at the forefront of their field of expertise are encouraged to attend.

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APPLICATION OF MICELLAR ELECTROKINETIC CHROMATOGRAPHY FOR DETECTION OF SILVER NANOPARTICLES RELEASED FROM WOUND DRESSING

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Introduction

The recent emergence of nanotechnology has provided a new therapeutic modality in case of silver nanoparticles. Dressings containing silver form the basis for the treatment of burns and wounds, either acute or chronic ones. The aim of the study was to examine silver release from the different wound dressings: commercially available (Atrauman Ag, Aquacel Ag) and experimental (FKDP-AgNPs) using MEKC. In order to characterize prepared keratin based wound dressing before and after its modification with AgNPs, a compositional analysis was conducted using energy dispersive X-ray spectroscopy. Nanosilver toxicity was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H tetrazolium test. Silver release from wound dressings was assessed using MEKC. The best separation was observed for MEKC in 20 mM borate buffer at pH 9 with 20 mM SDS addition. In vitro studies showed silver at higher concentration than 10 ppm exerted a toxic effect on fibroblasts isolated from diabetic mice versus NIH/3T3 and BJ cell lines (p 0.05). We observed silver was released more gradually from experimental FKDP-AgNPs wound dressing, in compare to commercially available wound dressings. The fast and low-cost method utilizing MEKC can be used in clinical practice to detect silver release from the wound dressings.

Materials and Methods

The particle size and zeta potential measurement

The particle size, along with the zeta potential of the synthesized AgNPs were estimated by Zetasizer, the Nano ZS (Malvern Instruments Limited, UK).

Silver release monitoring/determination

Examined dressings (Atrauman Ag, Aquacel Ag, FKDP AgNPs) and FBS (10 mL) were placed in a sterile 15 mL tube, and vigorously mixed for 72 h at 300 rpm. A 100 ul of each sample was collected after 1, 24, 48, and 72 h and analyzed by UV-VIS and MEKC. The experiment was performed in triplicate. The total content of Ag in all examined wound dressings was evaluated by F-AAS.

Results and Discussion

Zeta potential is a physical property describing a net surface charge of the NPs in the solution. Nanoparticles in solution repel each other, because they created a Coulomb explosion between the charges of the nanoparticles, which in effect prevents their agglomeration. It should be noted that the particles with values are more positive than +30 mV or more negative than −30 mV are considered to be stable. Zeta potential measurements showed the surface charge value to be –42.8 ± 6.65 mV. This negative surface charge is consistent with the electrostatic stabilization against aggregation and can indicate the long-term stability of nanoparticles. However, we observed a strong tendency towards their aggregation. This phenomenon was regularly observed during electrophoretic studies.

Capillary electrophoresis (CE) and MEKC can be used to separate nanoparticles based on their size different electrophoretic mobilities that are directly proportional to particles charge-to-size ratio. In the electromigration techniques, the main driving force is the electroosmotic flow, that depends on the electric charge of the capillary wall. When using fused silica capillary and BBS it was not possible to achieve satisfactory data for AgNPs separation. Furthermore, the NPs adsorption to the capillary surface and strong tendency to self-aggregation made the analysis practically impossible. Therefore, we proposed a solution of this problem, which is based on the addition SDS to the electrolyte used in the MEKC experiments. In our study it has been decided to implement the SDS surfactant due to several reasons, such as its high aqueous solubility, low CMC, small ultraviolet light absorption, and most importantly its availability at low cost. It was found that addition of SDS improved the peak separation as well as its symmetry, and in effect allowed successful determination of Ag release from examined wound dressings.

Conclusions

In summary, our study is one of the first to use MEKC to assess the release of silver nanoparticles and silver ions from wound dressings. Proposed MEKC procedure is simple, sensitive and useful for monitoring and detection of released silver nanoparticles from different dressings.

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CHANGES IN SELECTED PROPERTIES OF SILICONE BIOCOMPOSITES UNDER ACCELERATED AGING CONDITIONS

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Introduction

Unlike other polymeric biomaterials, silicones have unique properties (e.g., flexibility, low and high temperature resistance, chemical stability, gas and drug permeability, and low surface tension) that manifest in high biodurability and biocompatibility. However, these materials are characterized by low mechanical properties limiting their field of applications. Therefore, scientists are constantly developing silicone-based composites that will provide better mechanical performance while maintaining the required properties of biomaterials. Nowadays, researchers are turning towards acquiring composites incorporated with organic fillers owing to their adequate properties, such as availability, renewability, and simplicity of preparation compared to inorganic fillers. Multiple works reported the changes in the silicone matrix after incorporating it with different organic materials. Some works proved their favorable impact on mechanical properties [1,2], others - the opposite [3]. The results varied depending on the filler type, fraction, and structure. In literature, we can find many works studying the changes in silicone biomaterials due to subjecting to artificial aging with ambiguous results reported [4,5]. Nevertheless, limited papers study the effect of accelerated aging conditions on the properties of bio-based silicone composites. With that in mind, the authors took it upon themselves to develop new silicone biocomposites and investigate the changes occurring in the polymer matrix upon subjecting the materials to accelerated aging in a solution simulating body fluids.

Materials and Methods

An additional-crosslinking silicone was chosen as the composite matrix. Two herbs were incorporated: thyme (Thymus vulgaris) and sage (Salvia officinalis), in different mass weight ratios (5, 10, and 15 wt%). The composites were deaerated and prepared using gravity casting. The curing time was 24 h at room temperature and an additional hour at 60°C. After curing, thermal conditioning was conducted for 2 h at 80°C. The composites were subjected to accelerated aging in PBS solution for 2 and 60 days at 70°C.

Physical properties. Scanning electron microscopy (SEM) and laser diffraction (LD) were conducted to investigate the fillers' morphology.

Chemical properties

Fourier-transform infrared spectroscopy (ATR-FTIR) was carried out to indicate the changes in the materials' chemical backbone due to accelerated aging.

Mechanical properties

Hardness measurements using a Shore A durometer and tensile testing using a tensile machine were carried out. The crosshead speed was 500 mm/min. and stress at break and elongation at break were determined.

Results and Discussion

SEM micrographs reveal a well-developed surface of the fillers with visible trichomes and mesophyll cells. Particle analysis shows various size distributions, with sage particles being significantly bigger than thyme. The difference may be due to the fillers' different microstructure morphology and their individual behavior during the milling process.

The IR spectra of the aged composites reveals an increased peak corresponding to the stretching of the hydroxide bond, which is attributed to the chemical and physical effects of the water-based solution used for the aging process. This results from the hygroscopic character of the fillers. Moreover, the intensity of peaks at 782 cm^{-1} and 1004 cm^{-1}, characteristic of silicone, decreases as the number of aging days increases, indicating the materials' degradation process.

Hardness results show a diverse behavior of the obtained materials. Composites with thyme show an increasing hardness value with increasing mass fraction. The opposite situation is observed for composites with sage. After aging, hardness for most of the materials decreases. Stress at break drops significantly (up to 70%) after incorporating the fillers; however, its value remains to a similar extent after aging cycles. Analogous behavior is observed for the elongation at break values. The difference in the mechanical properties of the composites could result from the fillers' nature and their well-developed surface, which affects their wetting by the polymer.

Conclusions

Incorporating biofillers and undergoing accelerated aging alters the silicone's mechanical, physical and chemical properties to a varying degree. Such changes, depending on the application, may prove to be desirable.

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SURFACE TREATMENT IMPACTS PROPERTIES OF MATERIALS DEDICATED FOR FINGER IMPLANTS

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Introduction

Bone tissue has the ability to self-repair and regenerate in response to damage, however, huge bone defects occurring after tumour resection or traumatic fracture require external support in order to maintain the organ functionality. Finger amputation may result in failed replantation due to severe damage. Current research focuses on alternatives for surgical reconstruction by patient-specific, durable, biomimetic, bioactive and antibacterial implants for reconstruction of lost bone and joints [1–3]. Reconstruction of the hard tissue is planned by applying metal and ceramics additive manufacturing (3D printing). R&D focuses on selective laser melting (SLM) process development (optimization of laser power, scanning and powder layer properties) in order to achieve defined roughness and minimized defects from melting [4,5]. The goal of our work was to develop optimum mechanical properties, biocompatibility, and antibacterial activity due to surface modification of 3D-printed Ti64 (Ti-6Al-4V) and ATZ20 (Alumina Toughened Zirconia).

Materials and Methods

Nanoindentation tests were performed using Step 500 (NHT^3, Anton Paar). X-Ray Diffraction analysis was carried out using a Bruker D8 with CoKα filtered radiation. SEM analysis was performed using Scios 2 Dual Beam. For TEM analysis Tecnai G2 F20 (200 kV) FEG was used. The antimicrobial activity contact test was conducted according to ISO 22196, *E. coli* ATCC 8739 and *S. aureus* 6538P were used. Results were visualized as antibacterial activity index *R*. Direct cytotoxicity tests were conducted using Normal Human Dermal Fibroblasts (NHDF) C-12302 cells. Cells were incubated at 37ºC, 5% CO_2 for 24 hours. Live and necrotic cells were evaluated with laser confocal microscope (Exciter 5, Carl Zeiss) using propidium iodide and MitoTracker Green. Cell culture supernatant was evaluated for LDH (lactate dehydrogenase) levels (Cobas Integra 400, Roche).

Results and Discussion

Micromechanical tests indicated that electropolished and anodised specimens were harder and less elastic than other titanium samples due to TiO_2 layer. The ATZ20 was less elastic and harder than Ti64, on the other side HAp coating was relatively soft. XRD studies showed phase composition of tested materials. Electron microscopy studies confirmed diversity among surface topography (FIG. 1). Electropolished and anodized samples had no antibacterial properties, on the contrary hydroxyapatite coating killed bacteria efficiently (TABLE 1). Most of the tested samples did not show cytotoxic effect.

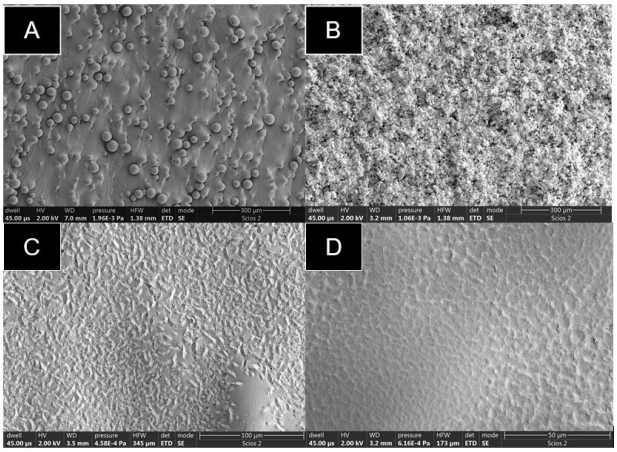

FIG. 1. SEM images of tested 3D-printed Ti64 surfaces: A – as built, uncoated, B – Hydroxyapatite (HAp) coating, C – anodised, D – electropolished.

TABLE 1. Antibacterial properties of tested materials. Material yields contact-killing properties if the *R* value is greater than 2 (orders of magnitude).

Material	Antibacterial Activity *R* against:	
	E. coli	*S. aureus*
Ti64 as built	2.6	0.38
Ti64 annealed	6.5	0.3
Ti64 as built + HAp	6.4	5.2
Ti64 annealed + HAp	6.4	5.2
Ti64 electropol. as built	0.2	0
Ti64 electropol. annealed	0.1	0
TI64 anodised as built	0	0
TI64 anodised annealed	0.1	0
ATZ20 + HAp	2.9	2.4
ATZ20 uncoated	1.6	0.3
ATZ20 + HAp/Zn	4.4	4.4
Ti64 annealed + Hap/Zn	4.4	4.4

Conclusions

This paper confirmed that surface modifications of Ti64 alloy and AZT20 ceramic may result in variety of mechanical, biological and antibacterial properties. Clearly, HAp coating shows the best biological properties. In brief, most of tested biomaterials had met basic finger implants requirements and are suitable for future *in vitro* studies.

Acknowledgments

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KERATIN BIOMATERIALS CONTAINING OPIOIDS ACCELERATE SKIN WOUND HEALING *IN VITRO* AND *IN VIVO* STUDIES

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Introduction

Chronic non-healing wounds is a major medical problem specially in diabetic patients. Pain associated with chronic non-healing wounds can be particularly difficult to manage. Therefore, effective wound care is a significant challenge for many professionals. Several different biomaterials like fibronectin, collagen and keratin gain much attention in biomaterials world. In this context, keratin biomaterials possess a unique set of properties, such as excellent biocompatibility, biodegradability, and bioactivity. The heterogeneous structure of the keratin scaffolds facilitates cell adhesion and allows surface modification with analgesic or antibacterial agents [1–3]. We evaluated an insoluble fraction of keratin containing casomorphin as a wound dressing in a full-thickness surgical skin wound model in diabetic mice.

Materials and Methods

Fur keratin-derived powder (FKDP) was coated with 0.1% solution of casomorphin (Caso) was prepared as described previously [1] and characterised in vitro using MTT test and in vivo in full thickness skin wound model as described previously [4]. Capillary electrophoresis was used for release of casomorphin from experimental dressing. Diabetes in mice (N = 20) was induced with 5 daily intraperitoneal (i.p.) injections of streptozotocin (80 mg/kg body weight) as described previously [4]. After 30 days, mice were considered as diabetic and surgical procedure was started. Two full thickness wounds were made on back mice, one serve as control – no dressing and the second was covered by keratin-casomorphin dressing. Wounds were photographed on day 0, 5, 8 and 15 post-injury. The rate of wound healing was evaluated as the difference between initial wound area and area on each post-wounding day and expressed as a percent of the initial wound area [1,5].

Results and Discussion

Casomorphin, was slowly released from the keratin dressing. In vitro study showed that keratin-casomorphin dressing is biocompatible, non-toxic, and supports NIH3T3 growth. In vivo experimentations demonstrated that keratin-casomorphin dressing significantly ($p < 0.05$) accelerates full-thickness skin wound healing during whole experiment. Wounds covered with keratin-casomorphin dressing underwent faster reepithelization, ending up with a thicker epidermis than control wounds, as confirmed by examinations. This investigated dressing stimulated macrophages infiltration, which favors tissue remodeling and regeneration, unlike in the control wounds in which neutrophils predominated as confirmed by immunohistochemical studies. Additionally, in dressed wounds, the number of microhemorrhages was significantly decreased ($p < 0.05$) as compared with control wounds.

Conclusions

This study showed that keratin dressing supplemented with casomorphin is safe and efficient, promoting skin wound healing in diabetic mice. We have proven that casomorphin was slowly released from the dressing, and in vitro and in vivo studies documented that keratin dressing is biocompatible, supports cell growth, and provides an immunomodulatory effect.

Acknowledgments

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SWELLING STUDIES OF CHITOSAN FILMS PREPARED UNDER DIFFERENT CONDITIONS

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Introduction

Over the past two decades, environmental protection has become an increasing challenge faced by the entire world. This has contributed to the development of research on the use of natural polymers, which are post-production waste, such as chitosan and its carboxymethyl chitosan derivative (FIG. 1). These polysaccharides are friendly to humans and the environment because they are non-toxic and biodegradable. They also have other extremely important properties, such as antioxidant and antibacterial character, and biocompatibility [1-2]. Thanks to this, they can be used in medicine, tissue engineering, and pharmacy. Research is conducted on the use of these polymers with the addition of cross-linking agents, in the form of mixtures with other polymers, and with the use of both methods [3-5]. These studies are aimed at improving the physicochemical, biological, and mechanical properties. This study was aimed to determine the influence of two cross-linking agent additions on the structure and swelling properties of chitosan materials. Swelling behaviour is one of the key properties of chitosan materials which characterize its applications for biomedical uses.

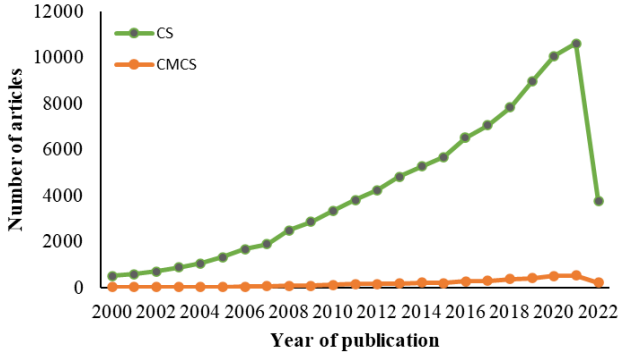

FIG. 1. Number of articles available in the Web of Science database on chitosan and carboxymethyl chitosan between January 2000 and June 2022.

Materials and Methods

Materials

High-molecular weight chitosan powder was purchased from Marine Fisheries Research Institute (Gdynia, Poland). Coffee acid was received from Pol-Aura (Dywity, Poland) and polyethylene glycol diglycidyl ether (PEGDE) was bought from Sigma-Aldrich (Poznań, Poland). All materials and reagents were of analytical grade and applied as received without further purification.

Methods

Chitosan was dissolved in 0.1M acetic acid. Then 2 films were poured. The rest of the solution was divided into four parts and the cross-linking agents were added: caffeic acid (15% and 20% (v/v)) and PEDGE (0.228 mM and 0.342 mM). The obtained solutions were stirred on a magnetic stirrer for 5 hours, then the solutions were poured onto PS plates. Films, apart from one chitosan film, were neutralized in 1% NaOH solution, and the films were dried again.

The following analyses were performed:
- swelling analysis in PBS solution at 37°C, incubation time 1, 4, 8, 24 and 48 h;
- ATR-FTIR analysis, VERTEX 70v FT-IR Spectrometer (Brucker Optics Inc), in the wavelength range between 4000 – 400 cm^{-1}, resolution of 2 cm^{-1} and 60 – times scanning.

Results and Discussion

Chitosan without neutralization was characterized by the highest swelling coefficients. The neutralization significantly reduced the swelling coefficients. Chitosan/caffeic acid films after neutralization were characterized by a slightly higher swelling factor than chitosan after neutralization. On the other hand, for chitosan/PEDGE films, the swelling factor was slightly lower than that of chitosan after neutralization. Based on the IR spectra, it can be stated that their neutralization caused the removal of acid residues in the chitosan films. The addition of caffeic acid (20% (v/v)) to chitosan significantly influenced the surface areas of the bands, reducing them. This proves a good cross-linking of the sample. In the case of chitosan/caffeic acid films, a correlation was observed between the crosslinking agents and the reduction of the surface areas of the bands. Generally, the surface area of characteristic bands decreases with an increasing cross-linking agent concentration. In the case of chitosan/PEDGE films, no such correlation was noticed. The smallest surface areas of the bands were recorded for the chitosan/PEDGE film with 0.228 mM of PEDGE.

Conclusions

The addition of cross-linking agents and neutralization significantly influenced the properties of chitosan films. The chitosan/caffeic acid with an addition of 20% (v/v) of agent film showed the highest degree of cross-linking.

Acknowledgments

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VITAMIN C ENRICHED BIOCOMPATIBLE AND BIO-DEGRADABLE FILM OBTAINED FROM MARINE-DERIVED NATURAL POLYSACCHARIDES

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Introduction

Among all types of skin wounds, great problems are caused by deep dermal traumas that increase the risk of infections and usually damage deeper layers of the skin, such as the dermis [1]. In contrast to the epidermis which may quickly regenerate in adults, dermis renovation is a more complicated process and in some cases may hardly occur [2]. The purpose of the research was to develop and comprehensively analyze a biomaterial based on chitosan and agarose with high potential use as an artificial skin substitute seeded with patient skin cells.

Materials and Methods

A thin polysaccharides film was produced by mixing chitosan solution prepared by dissolving chitosan in acetic acid solution and agarose solution prepared by dissolving agarose in sodium hydroxide at increased temperature. After combining both solutions, the non-toxic concentration of vitamin C was added and the contents of the beaker were thoroughly mixed to obtain a homogeneous mass, which was finally spread with a thin layer on the mold surface and air-dried.

Cell culture test. A cytotoxicity test on the produced thin film was conducted according to ISO 10993-5:2009. Additionally, human skin fibroblasts (BJ) and human epidermal keratinocytes (HEK001) morphology and their adhesion to the top surface of the sample were evaluated using confocal microscopy after fluorescent staining of cells nuclei, actin and vimentin filaments.

Biodegradation test. The degradation ability of the sample was investigated by immersing the biomaterials for two months in different enzymatic (collagenase and lysozyme) and non-enzymatic control (PBS) solutions. Collagenase solution simulated microenvironment of remodeling wound phase, whereas lysozyme solution mimicked chronic and infected wound microenvironment.

Results and Discussion

Conducted cytotoxicity evaluation showed that produced biomaterials (without vitamin C – CHN/A and with the addition of vitamin C – CHN/A + vit C) were non-toxic to the BJ cell line, since fibroblasts viability after 48 hours was comparable to the polypropylene control (FIG. 1). Furthermore, fibroblasts and keratinocytes co-culture were seeded on the biomaterial, and good cell adhesion and normal morphology were observed during confocal analysis indicating high biocompatibility of the produced film (FIG. 2). In the biodegradation assay, it was revealed that the obtained biomaterial was prone to enzymatic degradation. Changes in sample weight after degradation and surface topography during SEM analysis were noted (FIG. 3). High biocompatibility for both skin cell lines indicates a high potential of the biomaterial in case of its potential use as an artificial skin substitute. Additionally, its degradation ability in a biochemical environment typical for chronic wounds indicates its potential replacement by newly formed patient tissue.

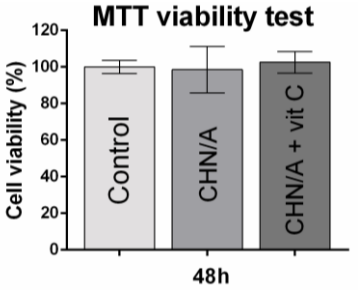

FIG. 1. MTT cytotoxicity test conducted according to ISO 10993-5 [1].

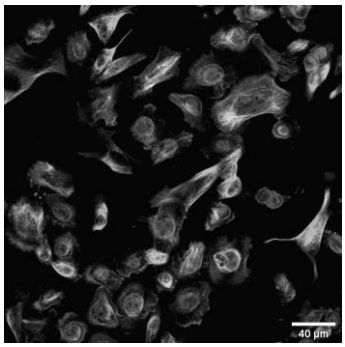

FIG. 2. Co-culture of BJ (spindle-shaped cells) and HEK001 (more round cells) on the surface of the CHN/A film [2].

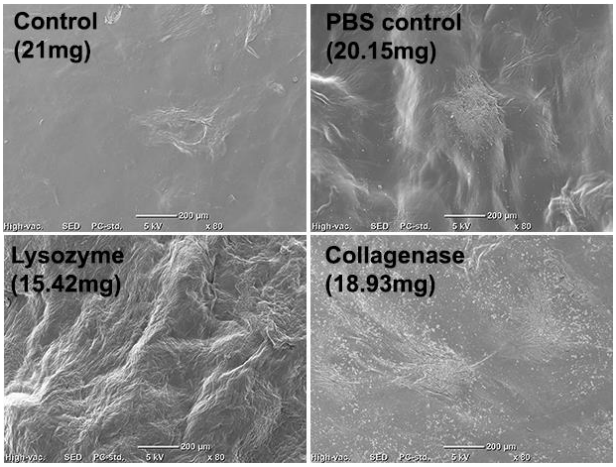

FIG. 3. SEM analysis of the CHN/A film surface after degradation in different incubation solutions [3].

Conclusions

Produced agarose/chitosan film is characterized by high biocompatibility and biodegradability confirmed in a simulated enzymatic wound environment. The favourable film adhesion properties for both skin cell lines make it a good candidate as a potential dermo-epidermal artificial skin substitute for wound healing acceleration.

Acknowledgments

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MULTICOMPONENT n-HA/ZnS/S-PEEK COATINGS ON ZIRCONIUM ALLOY: FABRICATION, MICROSTRUCTURE AND SELECTED PROPERTIES

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Introduction

Zirconium alloys are important metallic biomaterials generally used as bone implants. The Zr-2.5Nb alloy is one of the best known among biomedical zirconium alloys. Its importance in biomedical applications derive from outstanding properties, such as biocompatibility, high electrochemical corrosion resistance and relatively low elasticity modulus [1]. Although bioactivity of this alloy is poor. Therefore coatings are needed. The use of polyetheretherketone (PEEK) as coating materials is beneficial due to relatively low weight and high electrochemical corrosion resistance. It is a semi-crystalline, high-performance polymer with outstanding chemical and thermal stability, elastic modulus and stiffness similar to human bone, low density and good tribological properties [2]. One of the most successful ways of introducing bioactivity to PEEK is addition of bioactive agent such as hydroxyapatite (HA). The presence of HA induces bone formation, what leads to fixation to the human bone. On the other hand, HA has limited resistance to bacterial inflammation, which is among the most important implant failure factors [3]. A promising resolution to overcome this drawback is via the application of an antibacterial component in coatings, such as zinc sulfide. The ZnS is highly stable, antimicrobial and non-toxic. The sulfur content is a potential source that is required for the thermal sulfonation process of PEEK. Recent studies confirmed that sulfonated polyetheretherketone (S-PEEK) exhibits antibacterial properties and can exceed osteogenesis [4,5]. The aim of this study was to develop a duplex route based on electrophoretic co-deposition (EPD) and subsequent heat treatment for obtaining multicomponent n-HA/ZnS/S-PEEK coatings on the Zr-2.5Nb alloy, as well as to characterise their microstructure and selected properties.

Materials and Methods

Multicomponent coatings were developed by EPD on Zr-2.5Nb alloy discs. The HA nanopowder used for EPD contained elongated particles, with the average size of 10 nm. To prepare a suspension for the EPD of coatings, 1.5 g of PEEK704, 0.05 g of n-HA and 0.02 g of ZnS powders in 50 ml of ethanol and 5 vol. % of colloidal chitosan solution were mixed. The suspension was prepared gradually. Firstly, by dispersing the suspension containing PEEK and ZnS in an ultrasonic bath for 20 minutes. Then, by adding HA powder, magnetic stirring at 400 rpm for 10 minutes and dispersing for 10 minutes. Electrodes were immersed at a constant distance of 10 mm apart in the EPD cell. Coatings were deposited at a constant voltage in the range of 10-120 V with 20 V changes and a constant deposition time of 30 s. The specimens were heat treated in temperature of 450ºC for 40 minutes and cooled with rate of 2ºC/min. Thus obtained coatings were subjected to initial macroscopic observation. After that, the morphology and microstructure of selected samples was characterized by scanning and transmission electron microscopy (SEM, TEM). The structural studies were performed by X-ray Diffractometry (XRD) and Fourier-transform infrared spectroscopy (FTIR). Surface topography of coatings was examined by optical profilometry. Cross-cut tape test in accordance with ASTM D3359-17 was performed to investigate the adhesion of coatings to the substrate. Corrosion resistance of the coatings was examined in Ringers solution in 37ºC.

Results and Discussion

Zeta potential examination demonstrated that chitosan polyelectrolyte endorsed electrosteric stabilization of the suspension. Coatings obtained at voltages below 70 V were thin and inhomogeneous. Macroscopically homogeneous coatings were achieved for cathodic deposition in the voltage range of 70-110 V. Deposition with the use of higher voltages resulted in the presence of inconsistencies and pores. Therefore, the voltage of 90 V was used to obtain final coatings. Optimal deposition time was 30 seconds. Deposition for shorter period of time resulted in an unsatisfactory arrangement of deposits on substrate surface. Deposition longer than 30 seconds led to irregular surface of the coatings and increased number of pores. The heat treatment of temperature of 450°C and cooling allowed to achieve macroscopically homogenous coating with sporadic open porosity. XRD and FTIR showed an amorphous structure of PEEK and indicated its sulfonation. Coating was characterised by high surface area and considerable wettability with significant surface free energy (46.8 ± 3.1 mN/m) and wetting angle with water of 73.1 ± 3.5°. Coatings revealed high adhesion (the highest adhesion class (5B)) to the zirconium alloy substrate. The coated alloy demonstrated the ability to stimulate the apatite formation on its surface in simulated body fluid (SBF). The coatings exhibited a stable and significantly higher than not coated alloy substrate open circuit potential value of about 0.33 V.

Summary

Duplex treatment based on EPD and heat treatment allowed for the development of homogeneous multicomponent coatings on Zr-2.5Nb alloy substrates. As a result of heat treatment at a temperature of 450°C, a sulfonation of PEEK occurred in the coatings, leading to the formation of an amorphous PEEK matrix. The HA/ZnS/S-PEEK coatings demonstrated excellent adhesion to the alloy substrate, weakly hydrophilic character and had ability to stimulate apatite formation in the SBF and improved corrosion resistance of the alloy.

Acknowledgements

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ELECTROPHORETIC DEPOSITION, MICROSTRUCTURE AND BIOLOGICAL PROPERTIES OF MESOPOROUS SOL-GEL GLASS/ZEIN COATINGS ON Ti-13Nb-13Zr ALLOY

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Introduction

Titanium and its alloys are widely used in biomedical engineering because of their unique properties. The interest in them results mainly from their good biocompatibility, high electrochemical corrosion resistance, favoured fatigue strength and high strength-to-weight ratio. Despite its beneficial properties, titanium is a biologically inert material and tends to form biofilms on its surface. Therefore, in order to improve these properties, its surface is coated. In this work, composite zein-based coatings containing mesoporous sol-gel glasses (MSGG) with CuO and MgO were deposited on Ti-13Nb-13Zr alloy substrates by electrophoretic deposition (EPD). EPD is a versatile surface modification technique that allows the co-deposition of polymeric and ceramic materials [2]. Zein is a biodegradable, natural polymer which can be used as a biocompatible matrix of coatings [3]. MSGG are materials used in the regeneration of bone tissue, due to their high open porosity and large specific surface area [4]. Bioactive glasses are often doped with other elements to improve their biological properties, e.g. Cu (good antimicrobial properties) or Mg (stimulates the proliferation of osteoblasts) [5]. The aim of this work was to deposit MSGG/zein composite coatings with high adhesion to Ti-13Nb-13Zr alloy substrates, as well as to characterise their microstructure and biological properties.

Materials and Methods

The substrate material for the coatings was the Ti-13Nb-13Zr alloy, which was ground on abrasive paper with a gradation of 1200. The suspensions for EPD were prepared as follows: zein powder (200 g/dm^3) was gently added to the solutions of anhydrous ethanol (90 vol. %) and distilled water (10 vol. %), forming a zein solution, to which MSGG (70 SiO_2, 25 CaO, 5 P_2O_5) (reference material), MSGG-Cu (70 SiO_2, 25-x CaO, 5 P_2O_5 + x CuO (x=1-3)) or MSGG-Cu,Mg (70 SiO_2, 25-x CaO, 5 P_2O_5 + x CuO + 5 MgO (x=1-3)) (40 g/dm^3) was added. The EPD time of the coatings was 5 minutes at a current voltage of 5 V. The coating microstructure was investigated by scanning and transmission electron microscopy (SEM, TEM). The adhesion of coatings to the substrate was examined by tape tests in accordance with ASTM D3359-D. In-vitro cytotoxicity investigation of the coatings was performed with MG-63 cells (human osteosarcoma cell line). The MG-63 cells were prepared in cell culture polystyrene flasks and the medium was DMEM (Dulbecco's modified Eagle's medium) supplemented with 10 vol. % fetal bovine serum (FBS) and 1 vol. % penicillin/streptomycin (PS). The viability of MG-63 cells was assessed using the WST-8 assay. The observations of samples were made with a fluorescence microscope (FM). Live staining with DAPI and Calcein was used for the qualitative evaluation of cell morphology and viability. The antibacterial properties of glass powders and coatings were investigated against Gram-negative *E. Coli* and Gram-positive *S. aureus*.

Results and Discussion

The as-deposited coatings were homogeneous and had excellent adhesion (5B class) to the alloy substrate. The microstructures of coatings were dense and consisted of MSGG particles of various types and their agglomerates embedded in the zein matrix. The thickness of the coatings was ~10 µm. The evaluations of cell viability by optical density measurement showed deterioration after 24 h of incubation for all coatings in comparison to the titanium substrate. After 3 days, the cell viability decreased in all cases. Cell viability measurements coincide with the FM observations in that coatings showed a low number of cells on their surfaces. This was especially visible in coatings containing glass doped with Cu (MSGG-Cu), as well as Cu and Mg (MSGG-Cu,Mg), in comparison with the titanium substrate or control group. All types of MSGG particles showed antibacterial properties against Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria. After the first three hours of incubation, the reference MSGG particles showed the highest antibacterial properties. On the other hand, the antibacterial properties of coatings containing MSGG-Cu as well as MSGG-Cu,Mg particles increased with the time of incubation. The influence of coatings on the antibacterial properties was analysed with Alamar blue assay. All composite coatings showed antibacterial properties against Gram-negative *E. coli* and Gram-positive *S. aureus* in comparison to pure zein coatings.

Conclusions

The EPD allowed homogeneous composite MSGG/zein coatings to be obtained with high adhesion to the titanium alloy substrates. Their microstructures consisted of separate MSGG particles or their agglomerates in the zein matrix. A pronounced cytotoxic effect was observed for all coatings. The coatings showed antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* bacterial strains in comparison to pure zein coatings.

Acknowledgements

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RESEARCH ON THE PHYSICOCHEMICAL PROPERTIES OF TITANIUM NITRIDE COATING ON METALLIC BIOMATERIALS

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Introduction

Titanium nitride (TiN) is a ceramic material that has properties such as high hardness, high decomposition temperature, chemical stability at room temperature, and superconductivity. It is mainly used as a coating to reinforce other materials with similar properties. TiN shows encouraging blood tolerance properties with hemolysis rates near zero, so TiN coatings are used in cardiology, in ventricular assist devices for heart failure patients and in pacemaker electrodes. In neurology, TiN-coated electrodes are being studied to develop devices for chronic implants to treat, for example, spinal cord injuries. TiN coating is also used in dentistry for dental implants because of the excellent biological properties of TiN, such as limiting the release of cobalt-chromium-molybdenum (CoCrMo) ions, and because of the aesthetic aspect. Titanium alloys used for articulating surfaces require surface treatment to increase hardness and reduce wear. TiN coating has beneficial effects on biocompatibility and tribological properties of implant surfaces [1]. However, there are reports of third body wear due to delamination of PVD (Physical Vapour Deposition) TiN coating on Ti6Al4V, increased UHMWPE (Ultra High Molecular Weight Polyethylene) wear and cohesive failure of PVD TiN coating on CoCrMo hip implants in preclinical studies, and TiN coating puncture and fretting in femoral head recovery studies with TiN coating on Ti6Al4V. These adverse effects may be related to different coating processes of titanium alloys. The TiN coating process of titanium alloy joint surfaces should be optimized and standardized [2]. The overall properties of hard coatings, as well as their wear resistance and behavior, are strongly influenced by many key factors, including the substrate, deposition technique, temperature, and humidity, applied load, and test speed. There is no ideal choice for thin hard TiN-based coatings, and each operating condition should be considered individually [3]. In [4], titanium nitride coatings were deposited by CAE-PVD (cathode arc evaporation physical vapor deposition) method on two model metallic materials: 316LVM stainless steel and Ti6Al4V titanium alloy. It was found that the antiwear properties of TiN coatings deposited by this method are highly dependent on the mechanical properties of the substrate material. Studies have shown that the mismatch in elastic modulus between the substrate material and the coating is one of the most important factors determining the tribological properties of titanium nitride coatings. Although for both substrate materials, 316LVM stainless steel and Ti6Al4V titanium alloy, the TiN coating wore off during the test, the trybophilms formed on the substrate surface retained their protective function - an improvement in the wear factor K was observed for both substrate materials. Moreover, even if the coating was worn during the tribological test, the coefficient of friction remained reduced until the end of the measurement [5].

Therefore, the aim of this study was to determine the effect of TiN coating applied by PVD method on physicochemical and especially tribological properties of new generation titanium alloys.

Materials and Methods

The initial materials were two new generation alloys: Ti6Al7Nb and Ti13Zr13Nb. The 3 mm thick discs were cut from a 14 mm diameter rod and polished to a mirror-like surface. Next, the substrate was treated in an ultrasonic cleaner for 15 min in 96% ethanol and processed by magnetron sputtering system nanoPVD-S10A by Moorfield. Physical Vapour Deposition is related to deposition by magnetron sputtering. The system has automatic pressure control. Argon was used to generate the plasma for thin film deposition by gas phase deposition, while the precursor-target selected for modification was titanium nitride. The gas was introduced at a power magnitude of 25% and the table rotation rate was 3 J. The PVD method allows for a relatively low percentage of substrate surface coverage, while it has the highest coating deposition rate per minute. To determine the quality of the obtained coatings, several mechanical and physicochemical tests were carried out, including ball-on-disc abrasion tests and scratch test adhesion of the layer to the substrate. Scanning electron microscope (SEM) observations were also performed. Surface roughness was measured by a contact profilometer, wettability was measured by session drop technique and pitting corrosion resistance was measured in a Phosphate-Buffered Saline (PBS) environment.

Results and Discussion

The measured surface roughness on both substrates is comparable, as is the wettability. This is due to the same method of substrate surface preparation and to the achievement of high repeatability of the TiN layer. The abrasiveness, as well as the adhesion of the layer to the substrate, are strictly dependent on the type of substrate, so the results obtained differ. Observations by SEM confirm the achievement of a homogeneous coating. The results of corrosion resistance tests confirm the good biocompatibility of the material.

Conclusions

The results of the study allowed us to determine a wide range of properties of the TiN layer obtained on the substrate of new generation titanium alloys. The properties of the obtained coating depend on the substrate to which the coating was applied. TiN coating on titanium alloys, due to its biocompatibility and wear resistance, can be used, for example, for orthopedic implants.

Acknowledgments

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SURFACE MODIFICATION OF Ti AND Mg FOR BONE TISSUE IMPLANTS

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Introduction

Recently, the search and generation of new suitable solutions for orthopaedic implants' surface modification is required due to increasing number of bone replacement. Biomedical research's focus shifted from implant geometry to the functional potential of surfaces [1,2]. It is a fact that the most implants for bone substitution are predominantly made from Titanium (Ti) and Magnesium (Mg) alloys. Despite the high clinical success rates, their performance could benefit from improvements in: (i) controlling the corrosion process, (ii) shortening the initial healing period, (iii) enhancing osseointegration, and (iiii) decreasing the risk of implant-associated infections — a matter of increasing importance, given the mounting prevalence of multiresistant bacteria [3,4].
Our research aims to select the treatment for Mg and Ti-based materials to mimic bone structure and functionality.

Materials and Methods

Pure Titanium and Magnesium were used as a substrate. The samples were ground with wet sandpapers from 240 to 1200 to remove the natural oxide layer and ultrasonically cleaned with isopropyl and distillate water.
For Ti samples we used a combined procedure for modification of the surface of the commercially pure Ti which includes sandblasting, oxalic acid etching, and alkaline treatments with 5M NaOH. For Mg samples we applied plasma electrolytic oxidation (PEO) in silicate-based bath with calcium phosphate particles - stoichiometric hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP), obtained from Sigma–Aldrich (St. Louis, MO, USA). The electrolyte for PEO treatment was prepared with a solution of 10g/L Na_2SiO_3, 5g/L NH_4F, 10g/L NaOH, 4g/L HAP. The obtained surfaces were analysed by scanning electron microscopy (SEM), energy dispersive x-ray analysis (EDX), and contact angle and roughness measurements.

Results and Discussion

The osseointegration mechanism is generally defined as the adhesion of osteoblast cells to the metal implant surface. Therefore, our treatment was focused on surface development and functionalization.
Detailed analysis revealed that the complex approach of using sandblasting and acid etching produced a surface structure with highly developed Ti samples morphology on macro-, micro, and nano-levels (FIG. 1A). The alkaline treatment with sodium hydroxide additionally created a porous titanate layer with a trabecular structure. Notably, the alkaline treatment resulted in a hydrophilic surface layer, whereas other surfaces were hydrophobic. Moreover, the EDX analysis proved changes in the chemical composition. The obtained titanate layer had increased oxygen (semi-quantitative analysis). Incubation in simulated body fluid (SBF) suggested its ability to absorb calcium and phosphate ions. Hydrophilic surface indicates bioactivity properties and allows protein absorption and cell attachment.
Mg samples were anodizing in silicate-based electrolytes with HAP particles. The obtained coating had a porous structure. The surface and pores were filled with HAP particles (FIG. 1B). The removing of the top layer of the coating confirmed particle incorporation inside the layer. Moreover, the surface presents moderate roughness. All the obtained coatings were fluoride- and silicate-enriched layers.

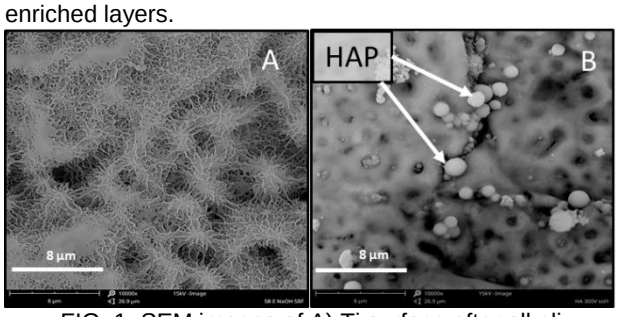

FIG. 1. SEM images of A) Ti surface after alkali treatment, B) PEO coating of Mg implant.

Promptly bone-like apatite formation on the implant surface proves a high level of bioactivity. In our study, mechanical and chemical treatment of Ti-based material coursed the formation of a bone-like biomimetic surface. The porous titanate coating had a significant promoting effect on the sorption of the phosphate and calcium ions. Meanwhile, an oxide layer on Mg samples could increase the corrosion resistance. The porous oxide layer creates a large surface area where osteoblast cells can be attached. HAP particles could be the source of the P and Ca for tissue formation.

Conclusions

Thus, our approaches, such as PEO with HAP particles for Mg-based implants or triple-step treatment for Ti-based implants, demonstrate a promising effect for further biomedical investigation. Obtained coatings, including Ca- or P- compounds, should improve the biocompatibility of implant surfaces with bone tissues.

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CYTO- AND GENOTOXICITY OF LASER-MODIFIED SURFACES OF METALLIC MEDICAL ALLOYS

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Introduction

Laser surface modification of medical grade metallic materials allows for far-reaching control of the response of cells in contact with these surfaces. We recently reported our observations made for Ti6Al4V alloy [1]. In this paper, we continue the analysis of cytotoxicity and genotoxicity for other commonly used medical alloys.

Materials and Methods

Four kinds of medical alloys: AISI 316L, Ti6Al4V, Ti6Al7Nb and CoCrMo. The surfaces of the samples were prepared as follows: Series A - surface after mechanical treatment (Ra 1.1 ÷ 1.2 μm), Series B - ground surface (Ra 0.5 ÷ 0.8 μm), Series C and D samples as in the A series, additionally laser modified using the Da Vinci 1300 Laser milling machine. Parameters: frequency 2.5 Hz and 10 Hz, pulse duration 1.2 ms and 10 ms, for Series C and D respectively.

Sterilization of the tested samples was performed with the use of a POL-EKO Aparatura hot dry air sterilizer, SRW 115 STD model, using a sterilization temperature of 180°C and a sterilization time of 45 min.

Normal primary human osteoblasts (NHOst) and chondrocytes (NHAC-kn) as well as neoplastic human osteosarcoma cells (line Saos-2) and chondrosarcoma (line SW1353) were used for cytotoxicity and genotoxicity testing.

Cytotoxicity of the tested materials was performed using the XTT test in accordance with the requirements of PN-EN ISO 10993-5: 2009 - "Biological evaluation of medical devices - Part 5: *In vitro* cytotoxicity tests".

The micronucleus test conducted with the InCell Analyzer system was used to test genotoxicity. Due to the lack of direct microscopic observation of the sample surface (high roughness), the tests were carried out with the use of extracts made in accordance with the guidelines of ISO 10993-12.

In the case of both types of assays, cells grown under optimal conditions were a negative control, while the positive control for cytotoxicity assessment was the administration of 0.01% Triton X100 solution to the culture, and for genotoxicity assessment, mitomycin C inducing chromosomal aberrations in the population of cells was used as a positive control.

The statistical significance of the differences was assessed by one-way ANOVA with the post-hoc t-Tuckey test for multiple comparisons.

The research was carried out in the Good Laboratory Practice regime.

Results and Discussion

The examples of CoCrMo and Ti6Al7Nb alloys surface modification seen under the SEM microscope are shown in FIG. 1. For AISI 316L steel, the results were similar to those for the CoCrMo alloy, and for the Ti6Al4V alloy, similar to those for the Ti6Al7Nb alloy.

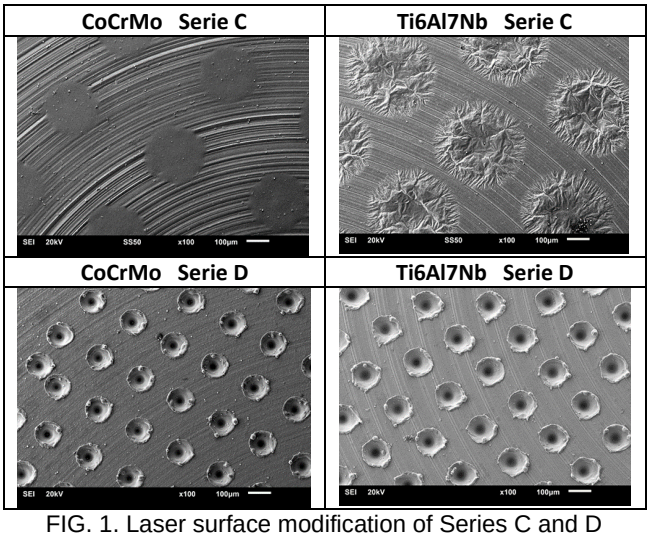

FIG. 1. Laser surface modification of Series C and D shown on the example of CoCrMo and Ti6Al7Nb alloy samples.

The mean values of the percentage of cell survival were statistically significantly different from the negative control, however, the difference did not exceed 30% in any case, which could be considered as a manifestation of cytotoxicity. Genotoxicity testing did not identify any sample tested as potentially genotoxic.

Conclusions

None of the tested samples, regardless of the type of surface modification, showed any signs of cytotoxicity or genotoxicity.

Acknowledgments

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SYNTHESIS AND CHARACTERIZATION OF AMPHIPHILIC POLYMER NETWORKS

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Introduction

Advanced biomaterials have become a powerful tool to enhance the diversity of applicability of various inorganic/organic, hydrophobic/hydrophilic and elastic/stiff materials in medical field [1]. Injectable biomaterials used for tissue engineering are designed to limit the invasive nature of their implantation, and thus can offer advantages in certain clinical applications [2]. Moreover, elastomeric properties and bioadhesiveness can make them suitable for soft tissue repair. Added value can be their biocompatibility or degradability making them suitable for tissue engineering applications [3]. Herein, we present the synthesis of amphiphilic polymer networks consisting fatty acid derived ester-urethane telechelic macromonomer (component A) being used in combination with PEGylated fibrinogen (component B) to create amphiphilic polymer networks and an increased biocompatibility. In order to induce adhesion to wet surfaces, a modified mussel adhesive protein (component C) was used in the final formulations. Finally, characterization of the hybrid components, including the study of a hydrolytic and enzymatic degradation the obtained amphiphilic polymer networks (hybrids) was investigated.

Materials and Methods

Components of amphiphilic polymer network are synthesized separately by methacrylation of dimerized fatty acid derived ester (Priplast 1838, Croda) (component A) and 3,4-Dihydroxy-L-phenylalanine (L-DOPA)(component C), and acrylation of PEGylated fibrinogen (component B). Crosslinked films were prepared as follows: photoinitiator 2% w/w (Omnirad 2022) was mixed with macromonomer, PEGylated fibrinogen and methacrylated L-DOPA with various ratios in ethyl acetate:DMSO (95:5). Residual solvent was evaporated under reduced pressure. Then, 1-mm thick films were produced by pouring the final composition onto glass plate and spreading with a steel applicator. The composition was then irradiated with a DYMAX Bluewave LED Prime UVA light source (λmax: 385 nm, 20 mW/cm^2). The water contact angle was measured by Krüss DSA100 for the hydrophilicity of the obtained films. Adhesive properties were assessed by peeling test according to ASTM-D2861 norm using an Instron 3366 testing system. Effect of L-DOPA functionalized surface was perfomed by Leica DMi8 fluorescent microscopy.

In order to investigate stability of the obtained hybrids, enzymatic and hydrolytic degradation has been performed. The polymer discs used in this study were cut from cross-linked polymer films using manual puncher. The disks were of 6 mm in diameter and weighted 15-20 mg. Each disc was immersed in 2 cm^3 of the medium. Each data point was an average of 4 samples. For examining the changes of degradation medium's pH, additional samples containing 0.1 g of material in 10 cm^3 of medium were prepared. During the test samples have been incubated and shaken in Heidolph incubator. Poly (ε-caprolactone) was selected as a reference material for enzymatic degradation study.

Enzymatic degradation was induced by lipase from *Pseudomonas cepacia* (Sigma Aldrich, Poznan, Poland). A solution of enzyme in Dulbecco's PBS buffer (Sigma Aldrich, Poznan, Poland) at 25 units/ml (pH 7.25) was prepared. Sodium azide (POCh, Gliwice, Poland) was added to prevent the growth of bacteria. Degradation was carried out at 37 ºC for 22 days and samples were taken for analysis at every 24, 48 or 96 h. In order to preserve the enzyme activity, degradation medium was changed every 48 h.

Results and Discussion

Chemical structure of the obtained component A and C has been confirmed by ATR-FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. PEGylation yield of fibrinogen determined by FTIR spectroscopy and colorimetric assays showed 88 and 72%, respectively. Water contact angle results showed that the introducing PEGylated fibrinogen and methacrylated L-DOPA increased the hydrophilicity (FIG. 1). The biological and adhesive properties assessment by fluorescent microscopy and peeling test indicated lack of cytotoxicity and good adhesion to wet surfaces.

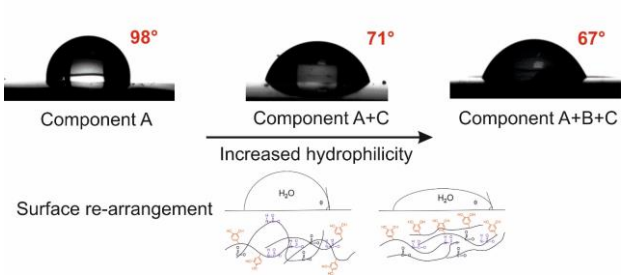

FIG. 1. Water contact angle values of the amphiphilic hybrids.

Conclusions

Adhesion strength of materials increased twice by introducing 5 and 10 wt.% of L-DOPA molecule. The infrared analysis of the polymers revealed the formation of new functional groups that derived from hydrolysis products of these networks such as poly(methacrylic acid), macromonomer precursor molecules and other derivatives of the of macromonomers having terminal carboxylic and hydroxyl groups. All discussed results confirmed the usefulness of considered flexible polymer networks for biomedical applications where controlled slow degradation is a desirable feature. Moreover, the results indicate high potential of these new materials for soft tissue engineering.

Acknowledgments

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DEVELOPING THE ELECTRICAL STIMULATION CHAMBER AIDING CARDIOMYOCYTES MATURATION

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Introduction

Developing mature tissue constructs that would aid in analysis of causes, development, and possible treatment (including tissue explants, drug screening, identification of risk factors) of various diseases is of great importance in current medicine. Within this field, usage of patient-derived cells is an interesting avenue as it allows working on the actual, even genetically burdened tissues.
In these applications, stem cells show great advantage over mature cells or cell lines, due to their high regenerative and differentiation potentials. In the adult human body, the so-called adult stem cells can be found in the cell niches where they serve as reservoirs for fixing damages tissues. These cells are multipotent, i.e. their differentiation is lineage-restricted, and can only be found in some, but not all tissue types [1]. In the heart, there is no stem cell niche able to recruit new cardiomyocytes (CM) [2]. Meanwhile, cardiac tissue remains one of the most important tissues to study, as its failure remains the most common cause of death [3] and cardiac toxicity of drugs constitutes the cause of around 20% drug withdrawals worldwide [4,5].
Because of that, different cell sources are needed to obtain cardiac tissue constructs *in vitro.* One possibility is the usage of human induced pluripotent stem cells (hiPSCs) which can be differentiated into desired cell lineages, including CMs [6]. Still, the greatest challenge that remains is to obtain a satisfactory level of the cell maturation [7].
As studies suggest, this could be improved by: electrical or mechanical stimulation, specific surface chemistry or morphology, or by bioactive compounds [8-11].
Combination of all of the above-mentioned factors is expected to produce the best outcome as it would be able to mimic the *in vivo* cell environment.
The aim of this study is to develop a new type of electrical stimulation chamber that would allow free modelling of electrical signal and its consecutive flow through electrically conductive and biomimetic scaffolds. In this preliminary analysis, the main goal was to verify the applicability of the system's elements (electrical conductivity and mechanical properties) and verify its cytocompatibility.

Materials and Methods

Printed circuit board was designed using the Autodesk Eagle software and fabricated by an external company. The casing of the electrical stimulation chamber and culture well molds were designed using Fusion 360 software and 3D printed. The cell wells were molded using Sylgard 184 (Dow Corning).
Elastic electrodes were obtained by modifying sylgard with carbon nanotubes (CNTs, Nanocyl 3150). The substrates were obtained from collagen type I (C9879, Sigma Aldrich), modified with oxidized CNTs [12] and glycerol (Sigma Aldrich). Chemical composition of the materials was identified using FTIR (Tensor 27, Bruker), mechanical properties were analyzed using the Inspekt Table universal testing machine (Hegewald – Peschke).
Ossila 4-point probe was used to evaluate the sheet resistance of the materials. Broadband dielectric spectroscopy (BDS) measurements were carried out using an MFIA 5 MHz impedance analyzer from Zurich Instruments and a custom-made cryostat [13].
Cytocompatibility of the PDMS nanocomposites was tested through the preparation of liquid extracts, in accordance with ISO 10993-5 standard [14]. Empty cell well served as a blank, and pure PDMS was used as a negative control. The tests were conducted on HEK 293T cells, cytotoxicity/cytocompatibility was established through MTT and LDH analyses.
Cytocompatibility of the collagen-based scaffold was evaluated by direct seeding of cells (HEK 293T and hiPSC-CM) on the surfaces of the materials. The morphology of cells was visualized using fluorescent staining while viability of HEK 293T was additionally analyzed via MTT assay.

Results & Conclusions

Introduction of CNTs grants the PDMs and collagen with electrical conductivity, with resistances of around 100kOhm – 300kOhm. The additives do not alter the materials' mechanical properties and there are no strong chemical interactions between the compounds that would negatively affect collagen's native conformation.

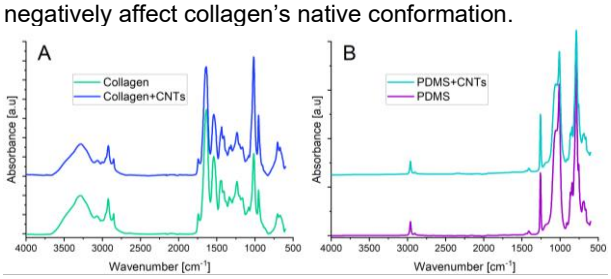

FIG. 1. FTIR spectra of the CNTs-modified samples: A) collagen foils, B) PDMS

All of the elements of the chamber are cytocompatible and can be used in further optimization of the novel device that would include electrical stimulation of hiPSC-CM cells.

Acknowledgments

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THE (POLYMER/TITANIUM(IV)-OXO CLUSTERS) COMPOSITE FILMS AS THE BIOMATERIALS OF ANTIMICROBIAL AND PHOTOCATALYTIC ACTIVITY

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Introduction

The growing threats from the spread of highly resistant microorganisms to widely used antimicrobial agents have led to increasing research on searching for new materials that reveal good antimicrobial activity. One of the research directions is using metal oxides with photocatalytic properties, especially titanium dioxide. However, the limitations in the practical application of TiO_2 are due to the wide bandgap resulting in the necessity of excitation mainly by ultraviolet light. The use of titanium(IV)-oxo complexes ($[Ti_aO_b(OR)_c(OOCR')_{4a-2b-c}]$ (TOCs)) may be an alternative to the materials basing on titanium dioxide as an antimicrobial agent. By applying the TOCs, we can control the $\{Ti_aO_b\}$ core structure, the type of carboxylate ligands, and the way their bonding with the core – which in turn has an impact on the size of the bandgap and can lead to its significant reduction [1-4]. As a result, the photocatalytic activity of these systems effectively increases in the visible light range.
In our works, we have focused on the composite materials research produced by the TOCs dispersion in the polymer matrix. The formed polymer + TOCs systems were spectrally characterized and also their photocatalytic and antimicrobial activity have been determined.

Materials and Methods

The multinuclear Ti(IV)-oxo complexes (TOCs) were synthesized using the standard Schlenk and solvothermal techniques. The (polymer + TOCs) composites were produced by the TOCs dispersion (c.a. 5-20 wt.%) in the polymer matrix. The manufactured composite samples were characterized by spectrally. Moreover, their physicochemical and mechanical properties, wetting, free surface energy and the ability to generate ROS were examined. The biological properties research of the produced composites let to determine their antimicrobial activity, cytotoxicity, and the possibility to causing allergies.

Results and Discussion

The photocatalytic properties and microbiological activity of TOCs were studied for (polymer + TOCs) composites produced by the oxo clusters dispersion (c.a. 5-20 wt.%) in the polymer matrix. In our experiments, the following polymers were used: polystyrene (PS), polyethylene (PE), polycaprolactone (PCL), and poly(methyl methacrylate) (PMMA). The photocatalytic activity of composite films (polymer + TOCs) irradiated with UV and Vis radiation was assessed based on the decolorizing processes of methylene blue (MB) solution [3-7]. We have noted direct relationship between the type of carboxylate group and the photocatalytic activity of the synthesized compounds (e.g. the weak activity was observed for complexes containing the $-O_2CR'$; R' = -PhCl and -PhBr, while the activity increase was detected for $-O_2CR'$; R' = $-PhNH_2$, $-PhNO_2$, -PhOH, fullerene, and aspirin). Moreover, the results of our investigations indicated for the impact of the $\{Ti_aO_b\}$ core structure ($\{Ti_3O\}$, $\{Ti_4O_2\}$, $\{Ti_6O_4\}$, and $\{Ti_6O_6\}$) on the shift of the absorption maximum towards the visible range. Obtained results suggested that the photocatalyst particles excited in the visible range would generate reactive oxygen species (ROS) as a potential antimicrobial factor. For this reason, an essential part of our investigations, which precedes the microbiological tests, were EPR measurements, aiming to detect the generated ROS and determine their identity.
The antimicrobial activity investigations of polymer + TOCs composites were carried out against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and yeasts of *Candida albicans* [5,6]. According to these data, all (PMMA + TOCs) composites showed high antimicrobial activity against all tested bacteria. In the case of tests performed on yeasts of *C. albicans*, biocidal activity was found for composite containing the $\{Ti_3O\}$ core and stabilized by 4-hydroxybenzoic ligand, as well as for complexes with $\{Ti_4O_2\}$ cores.

Conclusions

The TOCs structure modification, involving the functionalized carboxylate ligands introduction into their structure, allowed to control the size of energy bandgap and to shift the absorption into the visible range direction. The influence of the $\{Ti_aO_b\}$ core structure on the photocatalytic activity of TOCs has not been fully understood. The comparison of the oxo-complexes with $\{Ti_3O\}$, $\{Ti_4O_2\}$, $\{Ti_6O_4\}$, and $\{T_{i6}O_6\}$ cores suggests that $\{Ti_4O_2\}$ and $\{Ti_6O_6\}$ systems show the best photocatalytic activity. Our investigations revealed good antimicrobial activity of (PMMA + TOCs) composite samples (the 20 wt.% addition of TOCs was used). Moreover, obtained results suggest the dependency between the polymer kind used to produce the composite system and composite microbiocidal activity. An example is better bioactivity of composite samples produced using PMMA and PCL matrix compared to PS, PE one.

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POLYCATECHOLAMINE FUNCTIONAL COATINGS ON COLLAGEN-SEALED POLYESTER VASCULAR PROSTHESES

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Introduction

Collagen-sealed polyester (PET) prostheses are commonly used in reconstructive vascular surgery due to its self-sealing properties. Impregnation with collagen makes the PET prosthesis non-permeable for blood and thus ready for implantation without any previous preparation procedures as so-called pre-clotting of unsealed PET prostheses. However, the problem of early or late infection of vascular prostheses still remains the Achilles' heel of modern vascular surgery. Vascular graft infection occurs in 0.6 to 5% of patients after reconstructions in aorto-iliac level. Mortality in this group is estimated to be between 25-88% [1]. Moreover, 40-70% of the patients will undergo major amputation [2]. Rifampicin- and silver-bounded grafts help to prevent infections but their efficiency is far from satisfactory. Polycatecholamine-coating of collagen-sealed grafts could solve this problem because this platform allows to bind significant amount of antibacterial agents.

Materials and Methods

Polycatecholamines were deposited on collagen-sealed knitted polyester vascular prostheses via in situ polymerization in mild alkaline buffer. Gentamicin was attached to the graft surface by simple soaking, resulting in stable binding of antibiotic to catechol moiety. The graft structure and properties were studied via FTIR, XPS, SEM and contact angle measurement while mechanical properties – by compression measurement. Reaction with whole human blood allowed to evaluate clot forming ability of the grafts and blood hemolysis. Cytotoxicity and proliferation of human endothelial cells was tested in cell cultures while embryotoxicity and neurotoxicity – in Danio rerio model. Antibacterial activity and bacterial adhesion was evaluated on 4 bacterial strains (Gram-positive and Gram-negative). USP 4 compliant flow-through cell tester was used for evaluation of drug release profile and parameters.

Results and Discussion

Polycatecholamine-modified (as proven by SEM, FTIR, XPS and contact angle) graft exhibited comparable wettability and elasticity as pristine commercial graft. Simultaneously, collagen-sealing layer remained untouched to prevent blood leakage outside the lumen of blood vessel. Polycatecholamine-coated grafts were shown to bind 6 times more aminoglycoside antibiotic (gentamicin) than pristine graft. Approximately 25% of immobilized drug was released from the graft while 75% remained attached to the prosthesis surface. Modified grafts showed the reduced hemolytic effect, lowered toxicity against human endothelial cells and reduced toxicity in Danio rerio model. Overall, poly(L-DOPA)-coatings deposited on PET vascular grafts can effectively functionalize collagen-sealed prostheses without the loss of protein sealing layer and allow for antibiotics incorporation to provide higher safety in biomedical applications.

Conclusions

The results obtained in this study lead to the conclusion that polycatecholamine-coating on collagen-sealed knitted PET vascular grafts is a promising method of their functionalization. It may serve as a platform for antibacterial drug binding, simultaneously keeping the collagen sealing layer intact.

Acknowledgments

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SILANIZATION OF SPHERICAL ALUMINOSILICATES USING TETRAETHOXYSILANE (TEOS) AND (3-AMINOPROPYL) TRIETHOXYSILANE (APTES), TAKING INTO ACCOUNT PROCESS VARIABLES, I.E. TEMPERATURE AND HEAT TREATMENT ATMOSPHERE

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Introduction

Many synthetic materials are used in biomedical engineering, including metals, ceramics, polymers, and cements to heal damaged bone. Despite the variety of available solutions, a material is still being sought with properties similar to bone. One such solution is spherical aluminosilicate. It is a material formed during the combustion of hard coal in pulverized furnaces. As a result of high temperature and under the influence of surface tension forces (changes in surface energy), the aluminum silicates take a spherical shape, and the gases released in the earlier stage of combustion create a cavity inside the newly formed spherical grains. This is how an ultra-light filler is created. The use of microspheres as a filler will allow to obtain a composite material not only of much lower density, but also of greater stiffness and stability. Surface silanization will allow for the production of functional groups, which will translate into the strength of the produced filler for polymers used in biomedical engineering.

Materials and Methods

The modification process was divided into three stages. First, the aluminosilicates were etched in a fresh hot Piranha solution that had been prepared with H_2SO_4 and 30% H_2O_2 in a 3:1 volume ratio. The acid was mixed in a beaker with a flat bottom on a magnetic stirrer (time 10 min; speed 350 rpm). After 10 min, microspheres were added and treated with Piranha solution for 30 min (speed, 350 rpm). After 30 minutes the Piranha solution was decanted into a separate beaker. To remove residual acid, the powders were filtered (4 x 1000 cm^3) with deionized water under reduced pressure using a water pump and then washed with 2-propanol. The second stage of the modification process was silanization with tetraethoxysilane (TEOS) and with the use of (3-Aminopropyl) triethoxysilane (APTES). The modification with the use of TEOS was based on two types of acetic acid and nitric acid catalyst. The modification from TEOS was applied on two types of acetic acid and nitric acid catalyst. The process is carried out in 25 wt% ethyl alcohol solution, then addition of TEOS (5 wt% ethyl alcohol solution + 10 wt% TEOS). Nitric acid was added in one sample, acetic acid in the other was added until the pH was 4; stirred for 30 min (speed, 350 rpm) at 50°C. The powders after the process were washed. The modification with APTES consisted in soaking the samples in the 2-propanol suspension for 1 hour (speed, 350 rpm), and then preparing the suspension with APTES in the ratio (10:1). The powders were then washed and dried in an oven for 24 hours at 90°C. In order to verify the process, tests were carried out, i.e. scanning electron microscopy with X-ray dispersion spectroscopy (SEM-EDS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Thermogravimetric analysis (TGA) with a heating rate of 10ºC / min (40ºC-1000ºC).

Results and Discussion

In the presented research, the aluminosilicate microspheres were surface modified in order to develop them by means of the etching and silanization process. The structure of the microspheres in the SEM study showed surface development through the use of an etching and silanization process. X-ray analysis revealed peaks typical of cenospheres containing mainly aluminosilicate phases such as mullite and silimanite, and other smaller phases such as quartz. The remaining reflxes define the modification associated with the use of silane. FTIR analysis showed the presence of Si-O-Si and Si-CH_3 bonds.

Conclusions

Modification of the surface using the silanization process will allow for the development of the surface and the strengthening of the chemical bond in composites. Spherical aluminosilicates are a promising material that could be a contender for applications in biomedical engineering.

Acknowledgments

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PIEZOELECTRIC NANOGENERATORS TO ENHANCE SKIN WOUND HEALING

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Introduction

Epithelium in healthy skin establishes and maintains a constant electrical potential. When skin layers are damaged, their electrical resistance disappears and an electric field is created locally in the injured epithelium [1]. However, in chronic wounds, the endogenous electric fields are compromised or absent. In addition, diabetic skin and normal skin of aged patients evince a lower transepithelial potential, which could contribute to a delay of wound healing [2]. Chronic wounds affect millions of patients around the world, and it is estimated that this would increase steadily in the future due to population ageing. Thus, new therapeutic approaches are being developed to enhance the wound healing process. In recent years, some studies analyzed the effect of exogenous electric field to enhance wound healing [3]. When strained, piezoelectric materials are able to create an inherent electric field. The use of piezoelectric nanogenerators (NGs) could enhance skin regeneration due to a local electrical stimulation. Here, we evaluate the biocompatibility of ZnO and polyvinylidene difluoride (PVDF) piezoelectric NGs and their effect on the proliferation and differentiation of cell types involved in skin wound healing.

Materials and Methods

ZnO NGs were synthesized by hydrothermal growth on an AlN thin-film layer previously deposited on glass coverslips. The ZnO nanosheets thickness was of 22 ± 2 nm. PVDF nanofibrous membranes were deposited on an Au thin-film layer by electrospinning technique, obtaining a mesh of nanofibers with 110 ± 20 nm of diameter. Human keratinocytes (HaCaT cells), fibroblasts (NHDF) and endothelial cells (HUVEC) were used for *in vitro* experiments. Cell viability and initial cell adhesion was analyzed after 24 h in culture using Live/Dead Kit (Invitrogen) and phalloidin staining of actin stress fibers, respectively. Cell proliferation was evaluated using resazurin assay (Sigma-Aldrich). Differentiation and stratification of keratinocytes were analyzed by immunofluorescence of cytokeratin 10 and 14. Cytokeratin 14 is presented in a basal proliferating layer, whereas cytokeratin 10 is presented in upper layers. The type I collagen synthesized by fibroblasts was immunofluorescently stained and quantified after 7 days in culture. The maturation of endothelial cells was evaluated by immunofluorescence of von Willebrand factor and platelet-endothelial cell adhesion molecule (PECAM-1, CD31).

Results and Discussion

ZnO NGs arrays demonstrated to be cytocompatible for the three skin cell types. ZnO NGs allowed cell adhesion and spreading of all of them, and cell viability assay showed that they were not cytotoxic (FIG. 1A). In addition, the number of keratinocytes adhered to ZnO arrays was significantly higher than on glass coverslips controls. All three cell types were able to proliferate on ZnO NGs. Keratinocyte differentiation and stratification were analyzed by cytokeratin expression. After 14 days in culture, a monolayer of basal keratinocytes and a second layer of differentiated keratinocytes was observed. The quantification of the area covered by differentiated cells showed a significantly increase of cytokeratin 10-positive cells on the ZnO NGs samples. As for the fibroblasts differentiation, the collagen synthesis by cells seeded on ZnO NGs was higher by 50% than by the cells growing on control samples. The maturation of endothelial cells did not show significant differences between ZnO NGs and the controls.

For the PVDF NGs membranes, similar results regarding the cytocompatibility were obtained. Cell viability analysis did not show any cytotoxic effect of PVDF on keratinocytes, fibroblasts (FIG. 1B) and endothelial cells. The cells were able to proliferate on PVDF without significant differences compared to control samples. Regarding the keratinocyte differentiation, after 7 days in culture, a basal layer of cells was positively stained for cytokeratin 14, and few cytokeratin 10-positive cells started to appear in the second layer. Endothelial cells were able to maturate on PVDF NGs membranes. Additional experiments will be performed to quantify the collagen synthesized by fibroblasts growing on PVDF membranes.

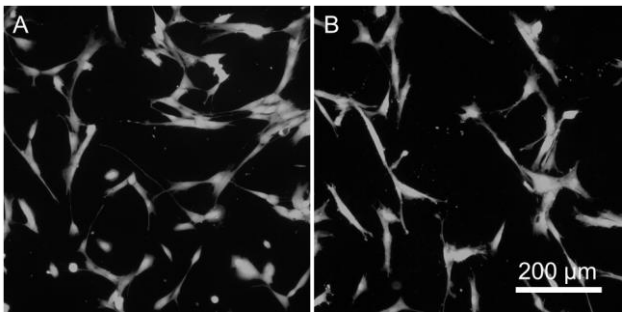

FIG. 1. Cell viability of fibroblasts growing on ZnO (A) and PVDF (B) nanogenerators. Calcein stained cells captured under Olympus IX71 microscope, DP80 digital camera.

Conclusions

In conclusion, piezoelectric ZnO and PVDF NGs allowed cell adhesion and proliferation, and proved to be cytocompatible for skin applications. Endothelial cells matured normally on both piezoelectric materials. Moreover, ZnO NGs enhanced the collagen synthesis of fibroblasts and the differentiation and stratification of keratinocytes. The *in vitro* results indicated that ZnO and PVDF NGs could be considered promising nanomaterials to enhance skin wound healing. Future experiments using dynamic cell culture and co-cultured systems will be performed to elucidate the potential therapeutic application.

Acknowledgments

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COMPARISON OF PHOTOCATALYTIC AND ANTIMICROBIAL PROPERTIES OF COMPOSITES MADE OF POLYMER AND TITANIUM(IV) OXO-COMPLEXES WITH $\{Ti_6O_6\}$ AND $\{Ti_6O_4\}$ CORE

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Introduction

Despite the great development of medicine and the growing awareness among the public about the dangers of microbial infections, we still hear a lot about infections that are difficult to contain, especially in hospitals. Such infections can have serious consequences for health, therefore new and effective solutions are constantly sought to stop the growth of microorganisms on various surfaces. A good solution may be to obtain materials showing photocatalytic activity in the field of visible light [1,2].
The aim of the research was to produce materials with photocatalytic and antimicrobial properties consisting of a matrix of PMMA and titanium (IV) oxo-complexes (TOCs) with 9-fluorenecarboxylic acid and $\{Ti_6O_4\}$ (1) or $\{Ti_6O_6\}$ (2) core.

Materials and Methods

Titanium(IV) oxo-complexes were obtained under an inert atmosphere, by mixing titanium(IV) isobutoxide with 9-fluorenecarboxylic acid in proportion 1:1 (Ti:COOH). Composite materials were obtained by introducing 10% or 20% TOCs into a poly(methyl methacrylate) (PMMA) matrix. The obtained composites were examined by Raman and IR spectroscopy, Raman mapping and also subjected to thermal analysis by TG and DSC methods. SEM EDX analysis confirmed the presence of TOCs in the polymer matrix. The photocatalytic activity was investigated by observing the degradation of methylene blue (MB) under UV and VIS light. Microbiological tests were carried out for the following bacteria: *Escherichia coli, Staphylococcus aureus* and *Candida albicans* yeasts.

Results and Discussion

The structure of TOCs (1) and (2) was confirmed by single-crystal X-ray diffraction. Both TOCs and pure PMMA exhibit the maximum of absorption in UV range but the introduction of TOCs into the polymer matrix results in the maximum of absorption also in the visible range (400 nm) what is especially seen in the case of $\{Ti_6O_6\}$ core. Taking into account the UV-Vis DRS spectra of both powders and composites, it was decided that the photocatalytic activity should be tested both in the UV and VIS ranges. The photocatalytic activity of TOCs with $\{Ti_6O_4\}$ and $\{Ti_6O_6\}$ cores was compared with the previously characterized TOCs with $\{Ti_3O\}$ (4) and $\{Ti_4O_2\}$ (5) cores. All of these materials are photocatalytically active but the best results were observed in the case of the $\{Ti_6O_6\}$ core. The estimation of antimicrobial properties proves that materials with 10 and 20% can reduce the number of bacteria: *Escherichia coli* and *Staphylococcus aureus.*

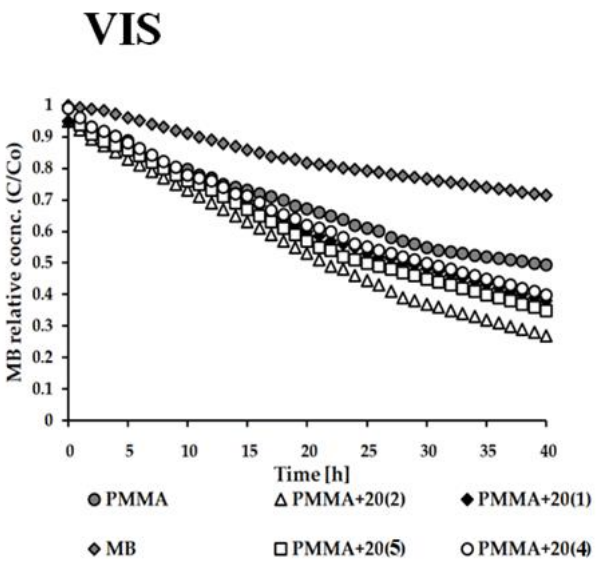

FIG. 1. Changes in the concentrations of the methylene blue (MB) solution as a function of time for composite materials PMMA + 20 (1), (2), (4), (5) irradiated with Vis light.

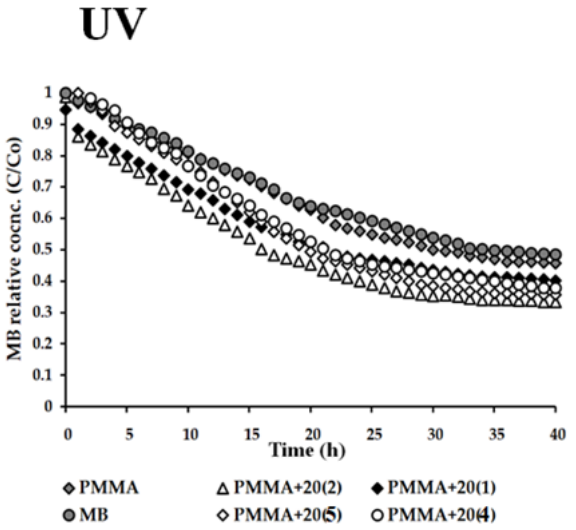

FIG. 2. Changes in the concentrations of the methylene blue (MB) solution as a function of time for composite materials PMMA + 20 (1), (2), (4), (5) irradiated with UV light.

Conclusions

The tests confirmed that composites with $\{Ti_6O_6\}$ core exhibit the best photocatalytic and antibacterial properties but it doesn't work against *Candida albicans* yeasts. Tested foils showed stronger biocidal activity against Gram-positive *Staphylococcus aureus* strains than Gram-negative *Escherichia coli* strains what results from the structure of these bacteria. In the future these materials can be used as antibacterial surfaces in everyday objects such as door handles, handrails, railings.

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NANOSTRUCTURED TITANIUM DIOXIDE LAYERS AS LOCAL DRUG DELIVERY SYSTEMS

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Introduction

Bacterial infections and their treatment are one of the biggest problems connected with implant failure. Currently used methods for drug administration – oral or intravenous – are insufficient since the dosages are usually too high and must be medicated very often [1]. The solution to the problems mentioned above is the use of drug delivery systems (DDSs) that may be applied locally [2]. Thus, the treatment would be more efficient and less toxic for the patient.

In the case of bone and dental implants, the most commonly used materials are titanium and its alloys due to their excellent mechanical properties and biocompatibility [3]. However, their functionality is limited due to the flat surface, which does not resemble the surface of bones. Thus, surface modifications are applied, e.g., to enhance the osseointegration. One of them is to synthesize a porous oxide layer on the surface of Ti via electrochemical oxidation of the metal [4]. It will provide enhanced surface area for cell adhesion and may be used as drug reservoirs from which a controlled and localized delivery of medicaments will be possible [5].

Materials and Methods

As starting materials, 2D and 3D Ti-based substrates were used. During the first step, anodic titanium dioxide (ATO) layers were formed on those surfaces via electrochemical oxidation (anodization). ATO layers were prepared under a constant voltage in the ethylene glycol-based electrolyte with fluoride ions and a small amount of water. Next, different drugs, i.e., ibuprofen, gentamicin or vancomycin, celecoxib, and the mixture of celecoxib with cyclodextrin, were loaded to the nanostructured TiO_2 layers. The loading procedure was adjusted to each type of the drug, while the amount of the loaded medicines was controlled by weighing the samples before and after the procedure. Some samples were then modified with cyclodextrin-polylactide (CD/PLA) by simple melting the polymer on the ATO layers. The drugs were released at 37 °C in the phosphate buffer solution (PBS, pH = 7.2) for up to one week. At each time point, the whole volume of the solution was withdrawn and replaced with the fresh portion of PBS. Finally, the amount of the released drugs from ATO layers was determined by using a UV-Vis spectrophotometer. In the case of celecoxib, thin layer chromatography (TLC) with densitometric detection was also used to determine the released drug. Finally, the release kinetics of each drug from the ATO layers were determined.

Results and Discussion

Anodization of Ti-based materials in the electrolyte with fluoride ions resulted in the formation of nanostructured ATO layers with vertically aligned channels. Depending on the applied voltage, the resulting pore diameters ranged from ~40 to ~80 nm. The length of the channels also varied due to the time of the process (from ~500 nm to ~3 μm). Thus, the capacity of the nanostructured layers varied, and different amounts of drugs might have been loaded inside such carriers. Also, by changing the substrate from 2D into 3D-printed, the amount of the loaded drug changed, and so did the release profiles (FIG. 1). Moreover, by depositing cyclodextrin-polylactide on the surface of ibuprofen-loaded ATO layers, we significantly slowed down the drug's release due to the inclusion complex between the drug and cyclodextrin (FIG. 1). On the contrary, when vancomycin was used as a model drug, we did not observe changes in the release profiles when CD/PLA was applied. Also, different release behaviors were observed for celecoxib mixed with cyclodextrin. These results show that release profiles from ATO samples depend not only on the carrier's capacity, but also the type of the drug and the polymer used to modify the surface.

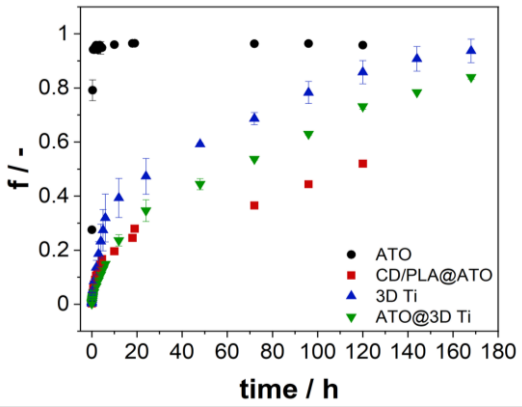

FIG. 1. Ibuprofen release profiles from different carriers: ATO layers (black dots); ATO layers covered with CD/PLA (red squares); 3D-printed Ti scaffold (blue triangle); anodized 3D Ti scaffold (green reversed triangle). Bars show the mean values of n=3.

Conclusions

ATO-modified Ti implants may act as reservoirs for different types of drugs. The release kinetics comprises two stages – burst release from the surface and a long-term release from the pores. The loading capacity depends on the length and diameter of the TiO_2 channels, while the release profiles differ for different types of drugs. Moreover, the release of medicines may be tailored by modifying the surface with polymers. Therefore, it may be stated that nanostructured TiO_2 may be used as local drug delivery systems for orthopaedic and dentistry applications.

Acknowledgments

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

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TRENDS AND PERSPECTIVES IN DESIGN AND FABRICATION OF THE REMOVABLE PARTIAL DENTURE FRAMEWORKS

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Introduction

Removable partial dentures (RPDs) are a group of prosthetic restorations that supplement missing teeth and damaged tissues of the prosthetic base in the maxilla and mandible [1]. They are a prevalent and economic treatment modality to enhance the quality of life of partially edentulous patients, characterized by the possibility of limiting the denture plate depending on the number and arrangement of the supporting elements related to the positioning of the supporting teeth, their quality and bone fixation [2,3]. Therefore, these dentures belong to the group of removable prosthetic restorations supported by periodontium and mucosa, where the advantage of one or the other component depends on the quality, distribution and quality of the pillars [5]. The transmission of chewing forces through the periodontium is considered the most physiological of due to the propri-receptive nature of neuromuscular control and the reduction of the component burdening the toothless alveolar processes. The aim of that kind of reconstruction is not only to restore the proper functionality of the stomatognathic system and aesthetics, but also to have a preventive effect aimed at maintaining the natural biological conditions and biomechanical balance for as long as possible. Initially, RPDs were produced from and cast metal alloys using the lost-wax technique. Digital production, computer-aided design and digital milling and printing manufacturing techniques have become prevalent and is gaining popularity due to its various advantages such as improved quality and faster manufacturing [6]. The specificity of the oral cavity environment determines the requirements to be met by prosthetic restorations - including high corrosion resistance and good mechanical properties. One of the basic materials used for the production of prosthetic restorations, with many advantages, are cobalt-chrome alloys [7,8].

Materials and Methods

The main aim of the research was to analyze the influence of surface modification (by applying zirconium oxide (ZrO_2) layers using the Atomic Layer Deposition method) of the physicochemical properties of the cobalt-based alloy (commonly used in dental prosthetics) - made by: SLS technology, CAD/CAM system and traditional casting method. The surfaces have been prepared in accordance with the principles of construction of removable skeletal dentures (RPD). A 50 nm thick zirconium oxide coating was applied to the prepared substrates. The main focus was on determining the effect of surface modification due to the release of metal ions, which may be corrosion products. It is particularly important due to the possibility of causing changes in the oral cavity, i.e. soft tissue inflammation, gingivitis, burning, metallic taste or even alveolar bone loss. The assessment of the permeability of ions to the artificial saliva solution simulating the oral environment was carried out using a spectrometric analysis sing the ICP-AES Type JY 2000 spectrometer.

Results and Discussion

The method of measuring the content of elements using ICP-AES is a comparative method. As a result of the tests, the content of individual elements was obtained [TABLE 1] with a standard deviation, which penetrated the solution from the sample, expressed in ppm. The concentrations of individual elements were converted in accordance with the formula (1) to μg/cm^2.

$$V_r \frac{ppm}{S} * 1000 = St[\frac{\mu g}{cm^2}] \quad (1)$$

where:
V_r - artificial saliva's volume (0.1 dm^3),
ppm - the concentration of the element expressed in ppm,
S - sample area (1.54 cm^2),
St - concentration of elements expressed in μg/cm^2.

TABLE 1. Ion permeability to the solution of artificial saliva.

CAST	**Co**	**Cr**	**Mn**	**Mo**	**-**	**-**	**Si**	**-**
	0.041	**0.028**	**0.011**	**0.009**	**-**	**-**	**0.003**	-
CAST ALD	Co	Cr	Mn	Mo	-	-	Si	Zr
	0.022	**0.017**	**0.008**	**0.004**	**-**	**-**	**no**	**0.005**
CAD/ CAM	Co	Cr	Mn	-	W	Fe	Si	-
	0.045	**0.031**	**0.015**	**0.01**	**0.009**	**0.012**	**0.005**	-
CAD/ CAMALD	Co	Cr	Mn	-	W	Fe	Si	Zr
	0.025	**0.012**	**0.009**	**-**	**0.006**	**0.008**	**no**	**0.004**
DMLS	Co	Cr	Mn	Mo	W	Fe	Si	-
	0.033	**0.025**	**0.010**	**0.005**	**0.007**	**0.009**	**0.003**	**-**
DMLS ALD	Co	Cr	Mn	Mo	W	Fe	Si	no
	0.017	**0.010**	**0.005**	**no**	**no**	**0.005**	**no**	

Based on the research, the impact of surface preparation on the number of metallic ions in the artificial saliva solution was found. Applying the zirconium oxide coating reduced the penetration of ions entering the tested materials into the solution, regardless of the manufacturing technology.

Conclusions

The optimization of the properties of materials used in dental prosthetics is currently a technological challenge in the face of the aging of society, civilization diseases and the need to ensure patient comfort. Precise technology, allowing structurally homogeneous elements to obtain, has a very significant impact on e.g. the initiation of corrosive processes.

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CURDLAN MODIFIED WITH SILVER AND Fe_3O_4 NANOPARTICLES VIA POLY(L-DOPA) DEPOSITS AS BLOOD-COMPATIBLE WOUND DRESSING CANDIDATES

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Introduction

Hydrogel biomaterials show many advantages in wound dressings design. We observe an urgent need to functionalize these types of constructs to fulfil additional functions. L-DOPA being a dopamine precursor in the biochemical synthesis pathway (with free carboxyl group in its structure) is beneficial surface functionalizer [1,2].
Previously we proved that it is possible to functionalize curdlan via poly(L-DOPA) layer using gentamicin molecule [3]. These results encouraged us to try to attach other molecules to curdlan structure via such layer. It was interesting for us to verify how the addition of these molecules would affect the functionality of the curdlan matrix. Bearing in mind the growing problem of antibiotic resistance, we decided to use nanoparticles with antibacterial potential as the alternative to antibiotic molecules. We selected four types of nanoparticles for that purpose. It seemed important to examine the mechanical parameters of the produced matrices and response of blood in contact with these matrices in terms of their future use as dressing materials for places exposed to mechanical injuries.

Materials and Methods

We introduced silver and Fe_3O_4 nanoparticles into curdlan alkaline suspension before its thermal gelling process, while we applied 2-LD-BG curdlan modification mode described elsewhere [3]. We produced control matrices with incorporated nanoparticles and matrices simultaneously modified with poly(L-DOPA) deposits and nanoparticles to determine the influence of poly(L-DOPA) on the properties of hydrogels. In the next step we evaluated the hemolysis and clot-formation of blood incubated with modified hydrogels following procedure described elsewhere [3]. Erythrocytes-released hemoglobin was estimated using reaction with Drabkin reagent. We also evaluated mechanical parameters of the matrices using EZ-Test XS machine. We determined resistance to compression and relaxation of the matrices.

Results and Discussion

The matrices modified that way showed high strength in mechanical tests in comparison with control hydrogel. Contact between wound dressing biomaterials and human blood is likely in wound treatment. Thus, it was important to define the impact of such biomaterials on blood compatibility. Some of the tested variants were found beneficial in the context of interactions with human blood - they did not induce hemolysis and did not significantly affected the formation of a clot after 30 minutes of contact with blood.

Conclusions

We assumed that addition of nanoparticles can be promising strategy for curdlan functionalization. The high mechanical resistance in combination with neutral parameters in contact with blood prompt us to conduct further and more advanced research to fully characterize the produced matrices.

Acknowledgments

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CATIONIC THIOLATED PVA MUCOADHESIVE BUCCAL FILMS FOR DRUG DELIVERY APPLICATION

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Introduction

In biomedical field, polymers play a vital role in advancement of drug delivery technology by providing controlled release of therapeutic agents in constant doses over long periods, cyclic dosage and tunable release of drug [1]. One such delivery system is the buccal film technology used for the systemic delivery of active pharmaceutical ingredients. Degradable polymeric drug delivery systems are an alternative for the management of drugs for the chronic inflammatory diseases [2]. Mucoadhesive based polymers are employed as drug carrier in buccal drug delivery system. Polyvinyl alcohol (PVA) is an attractive mucoadhesive biomaterial due to its high water content and biocompatibility. Moreover, abundance of hydroxyl groups present in backbone chain of PVA allows to readily modify with reactive chemistries for development of drug delivery systems. The aim of the research work is to develop PVA mucoadhesive buccal film for transmucosal drug delivery of Boswellia Seratta by modifying PVA with thiolation and incorporation of cationic moieties.

Materials and Methods

Mucoadhesive buccal films of Bowellia Seratta were prepared by solvent casting method. There are five different concentrations of L- Cysteine HCL and Cetyltrimethyl ammonium bromide (CTAB) incorporated into PVA solution (1% W/V) along with drug solution and Glycerol (0.5 ml). All films were formulated and named as PLC. Each solution was separately prepared and stirred 300 rpm at room temperature for twelve hours. Finally, the entire resultant solution poured into petridish and set aside 4-6 hours for drying. After drying, films were stored in sealed cover for characterization studies.

Results and Discussion

PLC5 films releases drug more uniformly than the other prepared films. There is not much difference observed between the films in terms of releasing the drug uniformly. From the in vitro drug release test results, we came to know that the PLC films release 44% approximately 70% of the loaded drug within 1 hour. And 45-50% of drug release was observed at the end of 30 minutes. A range of 35-89% of drug permeation is observed in the ex vivo drug release test. The wettability of film surface was characterized by using contact angle measurement. Based on the results obtained, we conclude that the hydrophilicity of prepared films is more than neat PVA film. FTIR results revealed that presence of thiol group in the PLC film.

Conclusions

The modification of PVA buccal film leads to formation of intra- and/or intermolecular disulfide bonds and improves the physical-chemical properties. The formation of disulfide bonds within thiol-containing polymers improves the stability, swelling behaviour and control release of drug buccal films.

Acknowledgments

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EXHALED AIR METABOLOME ANALYSIS FOR CHILDHOOD ASTHMA FINGERPRINTS IDENTIFICATION – THE PRELIMINARY STUDY

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Introduction

The diagnostic accuracy of tests used in early diagnosis of childhood asthma is limited and could benefit from the application of noninvasive omics technologies, both in clinical and population-based setting.
This study is focused on highly porous organic materials to identify metabolic content of exhaled breath [1] in asthmatic children and to develop an automatic classification method to measure metabolome changes including molecular mapping.

Materials and Methods

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

Results and Discussion

In asthmatic and not in control subjects the results of GC/MS showed the cluster of compounds including VOCS, SVOCS in the range of retention time from 12 to 30 min with the control peak of NOx, more apparent in asthmatic children (FIG. 1).

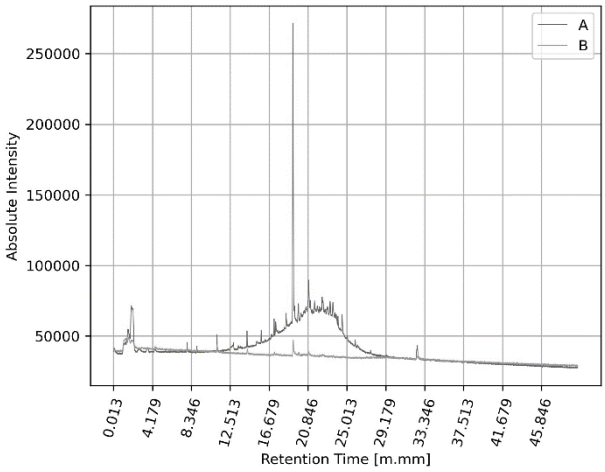

FIG. 1. Registered molecular fingerprint for healthy children B and for asthmatic children A.

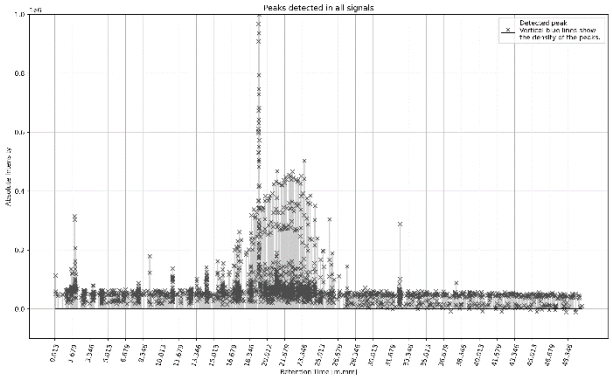

FIG. 2. The peaks indicated in the signal by the neural network recorded for asthma children

Conclusions

The use of GC/MS in analysis of metabolic content of exhaled air collected with novel porous polymeric material supported with neuron network peaks identification
(FIG. 2) seems to offer a sensitive and differentiating method supporting screening for childhood asthma in clinical and population-based setting.

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MODEL STUDY OF STRENGTHENING THE OSTEOPOROTIC VERTEBRAL BODY WITH BONE CEMENT WITH DISTRIBUTION CONTROL

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Introduction

Bone media is often used as a support for spondyloimplantology, less often on its own. The main role of these biomaterials is to strengthen the vertebral body, weakened, for example, by osteoporosis, or damaged, which alone cannot perform the supporting function in connection with an implant or a group of implants. In spine surgery, several groups of periosteal media are used, mainly silicone biomaterials [1-2] or PMMA-based cements [3-4], which are enriched with various additives [5-8]. The main disadvantage of cement application is possible set of complications resulting from uncontrolled leakages beyond the vertebral body area, which, according to the literature data, may be as high as 65% [9]. The aim of the study is to analyse the flow of selected cements - bone media in the lumbar body, prepared according to the manufacturer's guidelines, as well as to assess the possibility of influencing the reduction of adverse leakages through appropriate procedural steps and guidelines for in situ intraoperative management.

Materials and Methods

The study uses models of the lumbar bodies, developed on the basis of CTt data, taking into account the structural diversity of the bones, including the cortical tissue. The developed models were printed using the stereo-lithographic (SLA) method. A selected bone medium in the form of PMMA / SpineFix cement, dedicated to vertebroplasty of the spine, was used to fill the vertebral bodies. The cement was prepared according to the manufacturer's instructions and was fed in controlled manner with the same parameters on a special test stand. The injection into the bone through the supply canal was carried out in two variants: in the first group of samples, the insertion point was located in the central point of the endplate (1/2 of the vertebral body depth), in the second group it was shifted from the spinal cord, towards the anterior wall of the vertebral body (3/4 of the vertebral body depth). In both groups, two variants of the canal embedding depth before feeding were used: at ¼ and ½ of the vertebral body height. Industrial computed tomography CTt (16-bit image reconstruction algorithm with matrix consisting of 1024x1024 pixels) was used to assess the distribution of a given portion of cement in the vertebral body model.

Results

Spatial 3D analyses of cement distribution in the vertebral body model, carried out with the use of the Volume Graphics Studio Max 2.1 engineering software, showed that in the first group, with the central location of the injection point, cement spherically filled the intraosseous structures of the model. In the second group, fuller and less regular filling of the vertebral body was observed, which was associated with a lower bone density in the front part of the vertebral body. Each time, shallower placement of the distribution channel was associated with additional inflow of the cement under the lamina, which was more advantageous in the case of the accompanying reconstruction of the interbody space with the use of an interbody implant. The parameters and multi-directional geometry of the cement spread for each case were presented in a graphical form in a spatial arrangement according to the own assessment methodology.

Conclusions

The distribution of the medium in the vertebra model is influenced by various factors related to the parameters of the medium, the method of delivery, and the area of injection. The study proves the influence of the point location and the depth of the distribution channel embedding on the final flow of the medium. Which indicates that conscious intraoperative management can control the flow and reduce the risk of leakages causing intraoperative complications.

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TARGETED ADVANCES IN INNOVATIVE SPONDYLOIMPLANTOLOGY

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Introduction

The main message is to point to the development of BioMedEng as the most crucial element in the creation of progress to improve the life of society in general terms. It is sometimes assumed that this progress should be innovative. Here a doubt arises: is progress a "novelty" or is it an up-to-date use of existing knowledge? It can be assumed that innovation, is that which surpasses, and exceeds the "state of the art".

Aim

Following the path of implant innovation, successfully implemented in spine surgery using the example of the pioneering 3D-Ti-Truss stabilization, supported by the results of directed research efforts.

Material and Methods

The subject of this paper was a stabilization from the 3D-Ti-Truss family of products for surgical treatment of the spine with a printed implant of fusion type, made with the Electron Beam Technology (EBT) additive sintering of Ti6Al4V titanium powders. This is an example of the possibility of influencing the accelerated bio-mechanical support processes of the spine with triggered osseointegration through hybrid design, surface topography, biomaterial and manufacturing technology.
Studies of osseointegration, safety and functionality of the titanium alloy surface after 3D printing were carried out, among others, on human cells and on animals - "post mortem" evaluation after 3÷4 months of being implanted in the living organism. The ten-year clinical experience of using the "Ti-3D-Truss" biomaterial in bone surgery is presented.

Results

The Ti6Al4V ELI alloy, when used in the implant bearing-lattice structure and technological development of the surface (appropriate roughness/porosity), is a favourable biomaterial for a bone implant, which is confirmed in spondyloimplantology practice. Through the design, load-bearing properties conducive to bio-mechanics of stabilization were obtained, with a weight reduction of 50% on average. The manufacturing and processing technology allowed for an accelerated fusion by 35-40%. Based on observations, a mechanism was developed to explain the phenomenon of osseointegration to the implant surface (ivy-like mechanism of osseointegration - LC hypothesis), leading to the formation of an integrated implant/bone system.

Conclusions

Needs-driven research is a key element in achieving innovation and bringing a functional and safe implant into clinical practice. Sometimes advances are made without a complex research cycle, or research ascertains the "truth" to the acceptability of an invention. Patent, patenting is an example of indicating documented new technical solutions, usually practically useful.

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HYDROPHOBIC GENTAMYCIN – ADVANTAGES IN DRUG DELIVERY TO BONE TISSUE

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Introduction

Systemic delivery of antibiotics to bone tissue is not effective enough due to poor vascularity of the tissue. Therefore, different local drug delivery systems are investigated e.g. micro- or nanoparticles suspended in hydrogel or immobilized on the surface of the scaffold [1]. Nanoparticles (NPs) may be obtained from e.g. poly(lactic-co-glycolic acid) (PLGA) – a cytocompatible polymer with adjustable biodegradation kinetics. However, drug loading capacity of hydrophilic antibiotics (e.g. gentamycin (Gent)) in hydrophobic polymers is limited. Moreover, Gent release kinetics is often too fast. For this reason, we produced lipophilic complex of Gent (GentAOT) with bis-2-ethylhexyl sulfosuccinate (AOT) by ion-pairing. It allows to enhance encapsulation efficiency and provides more sustainable release kinetics for PLGA NPs without losing antibacterial properties [2,3]. Herein, we present a study dedicated to comparison between Gent-loaded NPs and GentAOT-loaded NPs in terms of drug loading capacity, release kinetics, morphology, and biological properties.

Materials and Methods

GentAOT has been obtained according to the recipe published earlier [3]. PLGA NPs were obtained using solid-in-oil-in-water emulsification. Briefly, PLGA (La:Ga ratio 85:15) from Polish Academy of Sciences, Zabrze, Poland was dissolved in dichloromethane (DCM, 2% w/v, Chemland) as well as either Gent (Merck) or GentAOT (0.2%; 0.4%; 0.6% w/v). The drug was dispersed by ultrasonic probe (3 min, 40% amplitude). Then, 3 ml of this solution was transferred to 20 ml aqueous solution of poly(vinyl alcohol) (PVA, 2% w/v, Mowiol 4-88, Merck). The emulsions were continuously stirred for DCM evaporation. The NPs were then collected by centrifugation, washed 3 times and freeze-dried. Morphology of NPs was characterized by scanning electron microscope (SEM), NPs size distribution and zeta potential were analysed by dynamic light scattering (DLS), encapsulation efficiency (EE) and drug loading (DL) were assessed by OPA assay. NPs manufactured with 0.6% drug were suspended in gellan-gum (GG) hydrogels. Briefly, GG (Gelzan, Merck) was dissolved in MilliQ-water at 90°C (1.4% w/v). Then, it was cooled to 50°C and NPs were added (4.44 mg/ml) loaded with Gent, GenAOT or mixed in equal weight. $CaCl_2$ was added for crosslinking (0.6% w/v) and Gent (0.1% w/v) for initial burst release. Release kinetics was measured incubating the formulation in the dialysis bags in PBS (4.44 mg/ml NPs in PBS or NPs-loaded hydrogel; 0.5 ml of formulation per 20 ml PBS). At several time points 2 ml of PBS was collected and measured by OPA assay. Collected PBS was replaced with a fresh one. NPs were compared in terms of cytotoxicity by culturing MC3T3-E1 cells in NPs suspensions (up to 1000 µg/ml) for 24 h. Cells were seeded in the 96-well plates (10,000 cells/well) and pre-incubated at 37°C and 5% CO_2 for 24 h. Then, medium was replaced with NPs suspensions. The viability of the cells was measured using AlamarBlue assay and live/dead fluorescent staining. Antibacterial properties of the formulations were assessed by Kirby-Bauer agar diffusion test with methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC BAA 1681). Holes of 6 mm diameter were excited in agar, and 100 µl of each formulation was added. After 24 h incubation at 37°C, inhibition growth zones were measured.

Results and Discussion

GentAOT was obtained successfully, which was proved by OPA assay (99.44 ± 0.04% of the substrate Gent underwent the reaction), and the product kept its bactericidal properties similarly to [3]. Manufactured NPs with drug showed sizes in the range 200-300 nm and all were slightly bigger than the empty ones (179 ± 1 nm). All the NPs were round and smooth on the surface, as shown by SEM observations. Empty NPs had the highest negative surface charge (-23.0 ± 1.4 mV) that was gradually reduced by the addition of Gent. On the other hand, all GentAOT-loaded NPs had a similar zeta potential of around -4 mV. GentAOT-loaded NPs showed superior drug loading capacity. EE was higher than 99% in all cases with resulting DL in the range of 10.79 - 22.54%. Gent-loaded NPs showed the highest EE of 32.22 ± 1.82% which resulted in DL of 5.33 ± 0.30%. The results seem to be more repeatable for GentAOT as well. Almost 100% EE for GentAOT was also observed in [2]. GentAOT release kinetics were also better. The initial burst release was prolonged for 5 days followed by sustained release, while Gent was the most intensively released only during first hours of incubation. GentAOT-loaded NPs showed better antibacterial properties. The inhibition zone were 33 mm for GentAOT-loeded NPs, and 16 mm for Gent-loaded NPs. Both types of NPs showed no cytotoxic effect against the MC3T3 cells at concentrations up to 500 µg/ml. At 1000 µg/ml Gent-loaded NPs also showed no cytotoxic effect, while almost all cells died in contact with GentAOT-loaded NPs at this concentration, suggesting that GentAOT may be more toxic than Gent. However, the concentration of drug was much higher there due to much higher DL.

Conclusions

GentAOT is a promising complex for PLGA-based drug delivery systems for the bone tissue. However, more detailed assessment of its biocompatibility will be required in the future.

Acknowledgments

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BESPOKEN BIOMATERIALS – SYNTHETIC STRATEGIES OF TAILORED NANOMATERIALS INVOLVING METALLIC NANOPARTICLES AND BIOACTIVE MOLECULES

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Introduction

According to the World Health Organization (WHO), if preventive efforts are not implemented urgently, infections related to resistant bacteria will be the leading cause of death by 2050. However, not only bacteria but also cancer cells may develop resistance to more than one therapeutic agent, when they are exposed to doses at an ineffective level of action for long periods of time [1-3]. Sustainable chemistry, involving natural compounds-based synthesis of metal nanoparticles (NPs), is becoming now an increasingly popular eco-friendly production method, even substituting chemical synthesis. In particular, phytochemicals (polyphenols) focus the attention of scientists based on their wide therapeutic potential with multi-faceted profile: antiproliferative activity towards various cancer cell lines, antimicrobial and anti-inflammatory activity. For instance, biopolymers (*e.g.*, chitosan (CS)), plant extracts (*e.g.*, *Rosa damascena* aqueous extract (RD)) as reducing and stabilizing agents are progressively used to synthesize metal NPs [4-7].

Materials and Methods

The emphasis in the developed syntheses was put on controlling the size of the metallic NPs by varying the synthesis conditions and thereby modulating their physicochemical and biological properties. All applied synthetic routes complied with the rules of Sustainable Chemistry. The morphology, size, crystal structure, and composition of the resulting materials were precisely investigated by UV–vis, ATR-IR, SEM, TEM, EDX, XRD, XPS, TGA, DLS techniques. The cytotoxicity of Au@RD NPs was evaluated *in vitro* on selected somatic cell lines: peripheral blood lymphocytes (PBML) and two cancer cell lines: acute promyelocytic leukemia (HL60), human lung adenocarcinoma (A549). To gain more insight into the mode of action of Au@RD NPs DNA damage in the treated cells was evaluated by comet assay, while the activity of initiator caspases 3 and 7 was measured by a luminescent assay. Antibacterial effect *in vitro* was studied towards the following bacteria strains: *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus paramycoides* by dilutions in the medium and disc-diffusion methods.

Results and Discussion

Energy-efficient, cost-effective, and eco-friendly synthetic routes of various types of tailored nanomaterials for therapeutic strategies of cancer and bacterial infections were optimized: *1*) Au NPs obtained with application of RD (Au@RD NPs), *2*) spiky bimetallic Au/Ag nanostars modified with RD (Au/Ag@RD NSs), *3*) Ag-Au alloyed NPs modified with CS (Ag-Au@CS NPs), and *4*) Au NPs modified with gentamicin and embedded in CS film (Au@gen NPs/CS). The selective cytotoxic action of Au@RD NPs towards cancer cells over somatic cells *in vitro* was demonstrated. Deliberate use of Au NPs in the resulting plant metabolites-based material was aimed at their potential dual function as a carrier and a contributor to overall activity (anticancer therapies, FIG. 1 a). While, the introduction of Ag atoms into bimetallic nanostructures was targeted to enhancing antibacterial action *via* unknown bimetallic mechanisms (antibacterial strategies, FIG.1 b-d)).

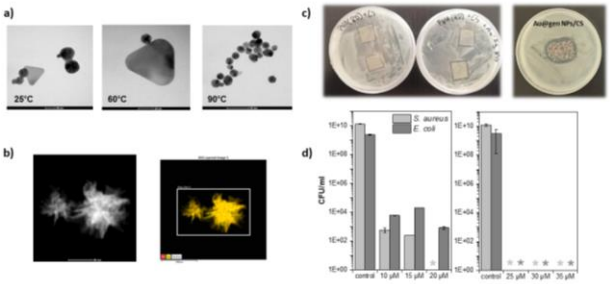

FIG.1 a) TEM images of Au@RD NPs, b) TEM and HAADF-STEM images of Au/Ag NSs, c) antibacterial activity of CS/PVA nanofibers impregnated with Au/Ag@RD NSs (left) and CS films enriched with Au@gen NPs (right), and d) Ag-Au@CS NPs d).

Conclusions

The resulting attractive hybrid materials combining bioactive components (metallic NPs, plant-derived metabolites, antibiotics) are alternatives to currently used ineffective and/or systemically toxic chemotherapeutics. Noteworthy, due to their hybrid nature, they give hope to overcome and learn about the mechanisms of drug resistance. Proposed herein efficient, facile, benign, and sustainable routes of development of biocompatible and biodegradable hybrid inorganic-organic assemblies emerge novel synthetic directions for the early application of such bespoken materials composed of metals and bioactive molecules.

Acknowledgments

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THE RGD ADSORPTION FOR BIOFUNCTIONALIZATION OF POLYURETHANE SURFACES

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Introduction

In contrast to bioinert materials, which are designed to prevent any interaction with the surrounding medium, bioactive materials come in contact with the environment and trigger a specific material–tissue interaction. For biomaterial applications, the interaction of a material's surface with cells is of critical importance. The interaction strongly depends on the physicochemical properties of the surface, i.e. wettability, roughness, or presence of functional groups, microstructure, and mechanical properties. Such properties determine cell attachment, as well as cell spreading behaviour, proliferation, and differentiation. The wettability of a solid substrate describes how the fluid adheres to its surface. Generally, most human cells preferably adhere, grow and proliferate on hydrophilic surfaces with a moderate contact angle (65°) [1]. In contrast, on hydrophobic surfaces, the cells exhibit a low proliferation rate. The biocompatibility, often represented in terms of wettability, has a general meaning and should be adjusted to specific applications, e.g. the different properties are desirable for orthopedic implants, blood contact devices and esophageal stents [2]. A key starting point for designing cell–adhesive polymeric materials is to activate surfaces with functionalities that promote the adhesion and survival of selected cell types involved in the wound healing or tissue regenerative process.

The proposed approaches for the improvement of biomaterials include enhancement of adsorption of specific proteins and material modification by immobilization of cell recognition motives (e.g. RGD, see structure in FIG. 1) to control the interaction between cells and polymeric substrates [3]. Protein adsorption at a biomaterial surface is currently extensively investigated both experimentally and computationally, yet still not fully understood. The aim of this study was to investigate the RGD peptide adsorption to polyurethane in the context of improving its surface biocompatibility.

Materials and Methods

Polyurethane (aromatic polyether polyurethane, American Polyfilm, Inc.) films were dissolved in tetrahydrofuran and deposited on the quartz crystal with the use of the spin coating technique. The adsorption kinetic of RGD (Adooq Bioscience, A15538) on polyurethane surface was followed with the use of Quartz Crystal Microbalance (QCM) QCM200, 5 MHz crystals QCM25, Stanford Research Systems set up. The system was equilibrated in protein-free PBS solution and after that RGD solutions of desired concentration was introduced (0,5 mg/ml and 1 mg/ml) into the flow cell. At the end of adsorption, the sample was again washed with PBS to remove weakly adsorbed (or present in liquid phase) peptide molecules. The detection of the RGD molecule was performed with the use of IR spectroscopy, Nicolet 6700.

The morphology of bare polyurethane surfaces and with adsorbed RGD were studied with AFM NanoWizzard 4 (JPK), ScanAsyst Fluid probes (Bruker).

Results and Discussion

Quartz crystal microbalance is an experimental method dedicated for measuring changes in adsorbed mass with high accuracy. All measurements were performed under dynamic adsorption conditions in the flow cell. The functionalized surfaces were thoroughly characterized by spectroscopic and microscopic methods.

The reference adsorption process was conducted for the model gold surface. The impact of the RGD solution concentration was examined. As expected, the overall adsorbed mass of the adsorbed RGD strongly depends on the solution concentration.

It was observed that the RGD adsorption on the PU surface is a slow process and the equilibrium is reached after ~10 hours (see the kinetic curve in FIG. 1). During this process, slow self-assembly of the peptides on the surface can be observed. With a calibration factor of 17 ng/cm^2 per Δf of 1 Hz, the saturation coverage amounts to 350 ng/cm^2 on the polyurethane surface (whereas for the reference gold surface is 300 ng/cm^2). The impact of topography on the RGD adsorption was investigated by AFM while the surface presence of RGD molecules was monitored by IR.

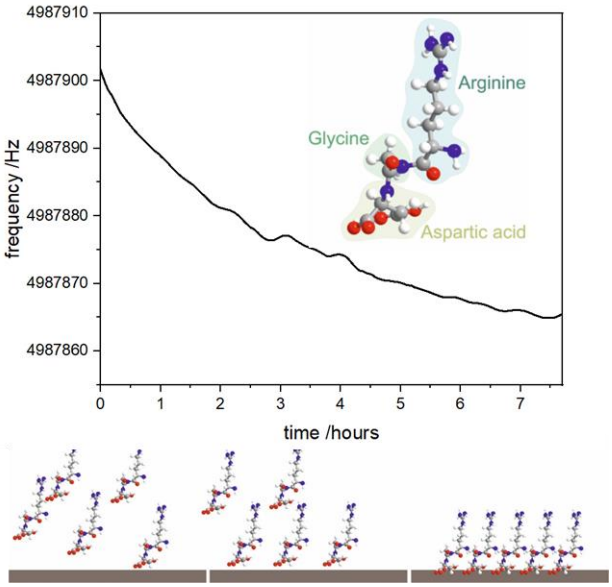

FIG. 1. RGD adsorption kinetic curve monitored by QCM and schematic representation of the process steps on the PU surface.

Conclusions

The surface of polyurethane can be decorated with adsorbed RGD peptides in a controlled fashion. QCM is a suitable method to be applied for monitoring the real-time adsorption of peptides as well as proteins' onto polymeric surfaces. The results provide the background for the development of polymeric biomaterials with improved surface biocompatibility. The results are discussed in terms of the improvement the cells' adhesion to polyurethane surface with pre-adsorbed RGD.

Acknowledgments

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WHEN BACTERIA MEET GRAPHENIC FLAKES – THE ROLE OF EDGES

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Introduction

Graphenic materials are extensively investigated in the field of materials science, chemistry, physics, biology, and medicine due to their unique physiochemical properties such as, large specific surface area, excellent mechanical strength, outstanding electrical, optical and catalytic properties and biocompatibility. In addition, the shape, size and morphology (defects e.g. edges) can also influence the properties of graphenic materials. These properties are essential for various biological applications including, antibacterial properties, biosensing, drug delivery, tissue and bone engineering to mention a few. The major issue with graphenic surface is that they are hydrophobic in nature which has to be adjusted for biocompatibility. This problem can be resolved by introducing polar oxygen groups on the surface, making it hydrophilic, enhancing dispersion in polar solvent, changing its electronic properties and improving the biocompatible of the material. The chemical nature of these groups however strongly depends on their surface location (flat surface, edges, corners). There are several reports how functionalized graphene (GO) with sharp edges can directly damage the cell membrane of Gram positive bacteria, thereby demonstrating the effect of edges on bacteria [1]. Although there has been a lot of investigations carried out in this field, still there is a gap in understanding the interactions of the interfaces of the graphenic materials (with different attachment sites i.e. flat surface and edges) with bacteria.
In this study, we investigate the effect of the edges in comparison with flat surface of oxygen functionalized graphenic flakes on bacteria adhesion. The research aims to fundamental understanding of bacteria - graphenic surface interactions which in broader perspective will provide the rationales for designing and optimization of carbon-based biomaterials surfaces with antibacterial properties.

Materials and Methods

The graphene flakes were prepared by ultrasonication of the graphene sheet and then modified with oxygen plasma. The obtained material before and after modification were characterised by AFM, SEM, XPS and work function measurements. The experimental results of graphenic surfaces functionalization were supported by molecular modelling of surface functional groups (DFT). The microbiological tests of bacteria attachment to the investigated graphenic surfaces were performed.

Results and Discussion

SEM and AFM images provide the information on the topography of the surface and showed that the plasma functionalisation of graphenic flakes was limited to the top surface keeping the bulk structure intact. In FIG. 1 a-b the typical morphology of the flat (low number of edges) and corrugated (high number of edges) graphenic surfaces used in the studies are presented. Since the plasma treatment introduce surface oxygen functional groups XPS reveals their chemical nature and concentration. The surface changes were also monitored by work function measurements (Kelvin probe). In general, the work function of graphenic surfaces increase upon plasma treatment, however, the effect strongly depends on the type of surface morphology. For flat surface the work function increased by 1.4 eV upon plasma treatment, whereas for the sample with high number of edges it increased only by 0.9 eV. To understand these findings, the DFT modelling of unmodified and oxygen functionalized graphenic surface was performed. The models used for calculations are presented in FIG. 1 c-d. It was found out that the surface locations of the oxygen groups (flat and edges) results in different dipole moments and thus various effects on electronic properties. It was also observed that the functionalization of graphenic surfaces is not permanent and partially decay in time due to the post plasma processes. The decay kinetics strongly depends on the surface morphology. This is also related to different stability of generated functional groups at the edges and at the flat basal planes FIG. 1 c-d.

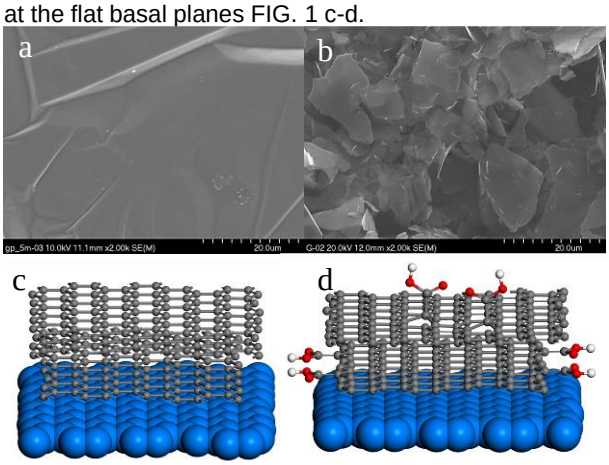

FIG. 1. SEM images of graphenic surfaces with flat (a) and corrugated (b) morphologies. The DFT models of unmodified (c) and oxygen plasma modified (d) graphenic surfaces showing various positions of surface functional groups (basal plane and edges).

As we previously found that the electronic properties of graphenic surfaces play an important role in the bacteria-surface interactions [2]. Therefore, the induced changes in graphenic surface properties (morphology, functional groups, electronic properties) were analyzed in terms of bacteria adhesion.

Conclusions

Based on the obtained results we can conclude that graphenic materials can be easily functionalized by oxygen plasma. The functionalization strongly depends on the surface morphology since the chemical nature of functional groups is different depending on the location. This is related to orientation of the surface dipoles and their effect on the electronic properties. It also results in different adhesion of bacteria to the investigated graphenic surfaces.

Acknowledgments

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PLASMA-ETCHED POLYMERS OF VARIOUS CRYSTALLINITY – CORRELATING BIOCOMPATIBILITY AND TOPOGRAPHY

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Introduction

Biocompatibility plays a major role in tissue-implant integration. The most important aspect that has to be taken into consideration while designing new biomaterials surface is the competition for the surface between eucaryotic cells and microorganisms, called the “race for the surface”. The outcome of this contest is critical for the patient since if bacteria attach to the medical device, they colonize it and produce an antibiotic-resistant biofilm. Therefore, the biomaterial surface has to be adequately prepared to promote cells adhesion and at the same time, limit the risk of bacterial infection.
It is not specified which material parameters are most critical for specific biological responses to surfaces. Since cells do not interact directly with bare materials surfaces but with conditioned ones, e.g. by proteins, peptides, and lipids. The factors that determine protein adsorption are the chemical composition, surface free energy, and roughness. The latter, especially nanotopography affects integrin-mediated cell adhesion and focal adhesion sites [1].
Polymeric materials used as biomaterials differ in terms of i.e. chemical composition, mechanical properties, and crystallinity. The surface properties of these materials can be modified with the use of plasma treatment. During the modification process, at first functional groups are formed. The subsequent stage after surface chemical modification is an etching, which begins with chain scissions of the backbone. As a result, substantial changes in surface morphology can be observed after exposure to plasma.
The study aimed to investigate the role of surface topography on biocompatibility and bacterial biofilm formation for a range of plasma-etched polymeric materials (amorphous, semicrystalline, crystalline).

Materials and Methods

For polyurethane (amorphous), parylene (semicrystalline) and polyethylene (crystalline) polymeric films were used. The XRD data were collected on a Rigaku Miniflex System and used for the crystallinity of polymeric materials. Plasma treatment was performed using Femto system (Diener Electronic). Changes in surface wettability were followed by contact angle measurements (Surftens Universal, OEG) and surface topography was determined with the use of an atomic force microscope (AFM, NanoWizard 4 XP Brucker).
The polymeric materials were also evaluated in terms of their biocompatibility (A529 cell line) and interactions with bacteria cells (*S. aureus*, *P. aeruginosa*).

Results and Discussion

Upon plasma treatment, the surface of initially hydrophobic polymers (Θ_W=90°) turned hydrophilic (Θ_W=20-5°). No changes in crystallinity were observed for the amorphous polyurethane, while for semicrystalline materials crystalline domains grew from 4 nm to 7 nm. Significant changes were also observed in terms of surface topography (R_{RMS}). The amorphous polyurethane after plasma etching became smoother, while for semicrystalline polymer nanotopography was formed (FIG. 1 A-B). The premise behind the observed effect is that during exposure to plasma, amorphous regions of polymers are preferentially etched, while the more compact crystalline domains are less affected as more etch-resistant [2].

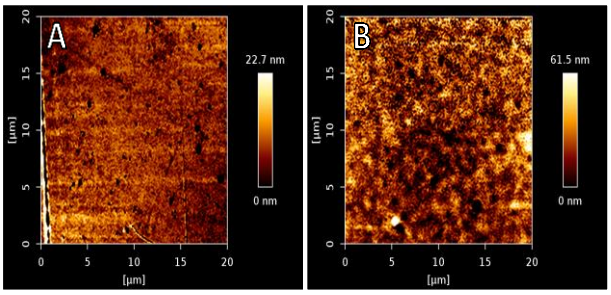

FIG. 1 Surface topography of unmodified (A) and plasma-etched (B) semicrystalline parylene.

Changes in surface chemistry and topography have also a positive impact on biocompatibility. Epithelial cells attachment to the plasma-etched surfaces was significantly higher than to the unmodified. Moreover, the plasma-etched surfaces of semicrystalline polymers were resistant to bacterial biofilm formation.

Conclusions

The obtained results reveal that the effect of plasma etching strongly depends on the polymer crystallinity. Amorphous materials smoothen upon exposure to plasma while the presence of the crystalline domains contributes to the nanotopography formation. A strong effect of surface chemistry and topography on biocompatibility and biofilm formation was observed.

Acknowledgments

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BIOMATERIAL COMPOSED OF CURDLAN, WHEY PROTEIN ISOLATE, AND HYDROXYAPATITE FOR OSTEOCHONDRAL TISSUE APPLICATIONS

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Introduction

Osteochondral defects are very common, pathological lesions in orthopaedic patients. Because they include not only cartilage but also subchondral bone, their repair requires the use of substitutes, which can replace both tissues simultaneously [1]. The desired biomaterials for the repair of osteochondral defects should primarily be biomimetic, i.e., their composition and properties should be comparable with natural osteochondral tissue. For these reasons, bi- and multiphasic scaffolds composed of natural polymers and ceramics gain increasing attention in the treatment of osteochondral defects [2,3]. The aim of this study is to present structural, mechanical, and biological properties of novel biphasic biomaterial composed of curdlan, whey protein isolate (WPI), and hydroxyapatite (HAp) for potential use in osteochondral tissue engineering.

Materials and Methods

The biomaterial was fabricated according to procedure described in details in Polish Patent no. 240639 entitled "Biphasic biomaterial based on curdlan and hydroxyapatite (HAp) for regeneration of osteochondral defects and the method of its preparation" [4]. The macrostructure, microstructure, and topography of resultant scaffold were evaluated using stereoscopic microscope, field emission gun scanning electron microscope (FEG-SEM), and optical profilometer, respectively. Young's modulus was determined during compression tests. Swelling ability of the biomaterial was assessed in the presence of 0.9% NaCl solution. Cytotocompatibility of scaffold was estimated towards two types of stem cells, namely human adipose tissue-derived mesenchymal stem cells (ADSCs) and human bone marrow-derived mesenchymal stem cells (BMDSCs). Cell adhesion and differentiation were assessed by confocal microscope observations, while cell proliferation was assessed using resazurin-based assay.

Results and Discussion

Developed fabrication method allowed to obtain novel curdlan/WPI/HAp biomaterial with two distinct but integrated phases, i.e., biphasic scaffold. Macroscopically, fabricated biomaterial was very similar with osteochondral autograft (harvested during standard mosaicplasty) (FIG. 1). Moreover, it was demonstrated that the top layer of the biomaterial composed of curdlan and WPI (so-called cartilage layer) possessed different structure (more smoother) than bottom layer composed of curdlan, WPI, and HAp (so-called subchondral bone layer). The compressive test indicated that Young's modulus of the top layer of biomaterial had very similar value compared to those of natural cartilage. In turn, the bottom layer of scaffold exhibited lower Young's modulus compared to subchondral bone. Liquid uptake test demonstrated that obtained biomaterial had very low swelling ability, which indicates that its potential use during surgical intervention is not limited. Cell culture experiments showed that both phases of the biomaterial promoted stem cell adhesion as well as proliferation, while bottom layer (enriched with HAp) was more suitable for cell colonization compared to the top layer. Differentiation assay demonstrated that biomaterial possessed chondroinductive and osteoinductive features *in vitro*, because both phases of biomaterials supported chondrogenic and osteogenic differentiation of stem cells (FIG. 2).

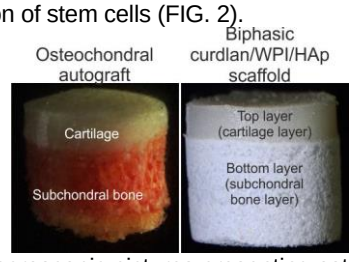

FIG. 1. Macroscopic pictures presenting osteochondral autograft and novel biphasic curdlan/WPI/HAp scaffold.

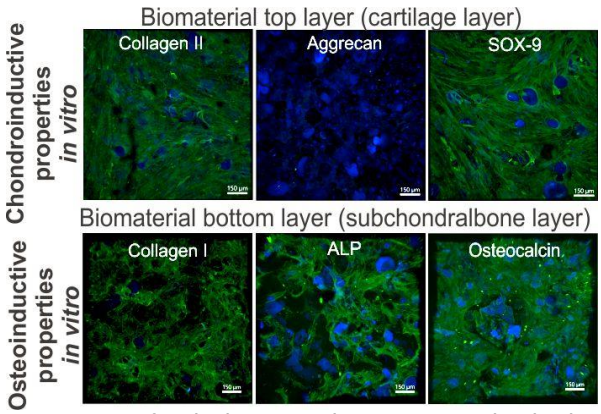

FIG. 2. Confocal microscope images presenting *in vitro* chondroinductive and osteoinductive properties of developed biomaterial.

Conclusions

This study demonstrated that novel scaffold composed of curdlan, WPI, and HAp was biomimetic nature compared to natural tissue. Performed *in vitro* research indicated that such biphasic biomaterial may be considered as promising scaffold for osteochondral tissue engineering applications.

Acknowledgments

This study was supported by the Ministry of Science and Higher Education in Poland within the DS2 and DS341 project of Medical University of Lublin, Poland as well as the Ministry of Health of the Czech Republic (grant No. NU20-08-00208).

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NMR-BASED AND RHEOLOGICAL SUPPORTED QUANTITATIVE ASSESSMENT POLYMERIZATION RATE OF BIOPOLYMERS (GELMA)

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Introduction

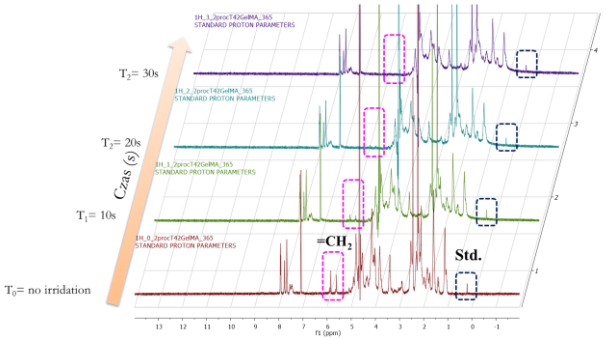
Recently, chemically modified and photocuring biopolymers ware found to be one the most promising biomaterial for reconstructive medicine [1-3]. Commonly used representative of this class of compound regarding accessibility and biocompatibility, is methacrylated gelatine. Although, numerous conducted studies, GELMA-based bioinks, suffer from lack of quantitative assessment of polymerization rate of methacrylated group. Therefore, we used NMR technique as the most reliable tool to establish optimal polymerization conditions for deeper understanding of photocuring process and reduce negative implication of UV light and photoinitator to living cells. Moreover, parallelly conducted rheological measurements confirmed NMR data.

Materials and Methods

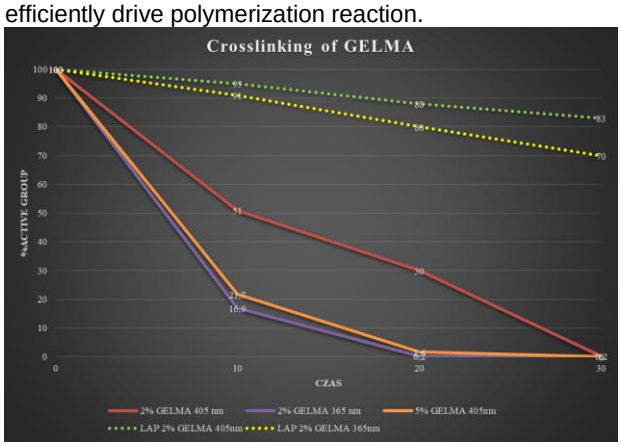
Applying commercially available, well defined GELMA polymer we conducted ^{1}H and ^{31}P NMR (700 MHz) measurement in strictly controllable photocuring conditions. All studies were carried out in terms of various variables encompassing both concertation of biopolymer and photoinitor (LAP), type of photoinitator, UV-Vis exposition time and wavelengths. Obtained data, proceded by Fourier transformation was analysed using classical integrations of signals originating from methacrylated group of GELMA (^{1}H NMR), photoinitiator and standards (both ^{1}H and ^{31}P NMR).

Results and Discussion

Performed analysis revealed that almost full reducing the methacrylic signal intensity as well as signal originating from photoinitiator (LAP) takes place significantly faster than previous reports about crosslinking of GELMA claimed. Worth noticing is that about 80% of methacrylic group disappeared after first 10s of UV light irradiation on sample and additional 10s of photocuring of polymer led to just 18% reduction active group signals. That observations strongly support theory that polymerization is higly dependent on concertation of sample.



Moreover, insightful analysis of ^{31}P NMR spectra showed that during irradiation process leading to full crosslinking of GELMA only 20% of initial photoinitator is sufficient to efficiently drive polymerization reaction.



Conclusions

Performed experiments and obtained data stand in opposition to common knowledge about crosslinking of methacrylated biopolymers and directly prove that whole process need to be revaluated and study deeper to get biomaterial science more proficient.

Acknowledgments

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

Conflicts of Interest

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

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FABRICATION OF HYBRID COATINGS FROM POLYCAPROLACTONE OBTAINED VIA ELECTROSPINNING

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Introduction

Modifying the surface of biomaterials is one of the ways of improving their biocompatibility and/or endowing them with features that facilitate the regeneration of surrounding tissues. The biomimetic fibrous surface structure is a solution to ensure effective connection of the implant with biological structures. In the case of fibrous materials, the biological response is influenced by both the geometry, size and the fibers orientation and by their physical and chemical properties. In this study, the surface of metal biomaterials used in cardiosurgery was modified with hybrid fibrous covers made of PCL polycaprolactone. Depending on the applied substrates and the parameters of the fiber application process, it is possible to obtain a coating with locally variable properties. The research employed electrospinning which is an effective method of obtaining fibers from polymer solutions. The hybrid structure of the obtained nonwovens can be created in two ways: via simultaneous spinning of the layers with needles located next to each other or on opposite sides of the collector, and via sequential spinning layer by layer (FIG. 1). In order to obtain nonwovens with evenly distributed hydrophilic and hydrophobic areas, the method of simultaneous spinning in a system of two parallel oriented needles was selected.

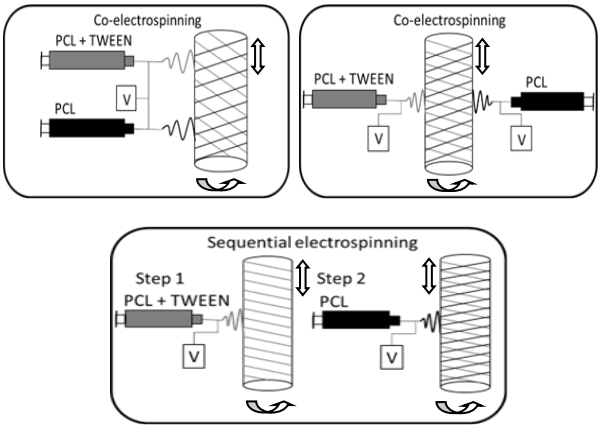

FIG. 1. Schematic diagram of electrospinning process - co-electrospinning and sequential setup.

Materials and Methods

Polycaprolactone PCL with a molecular weight of 80 kDa from Aldrich Chemistry. PCL was dissolved in a mixture of dichloromethane (DCM) and dimethylformadide (DMF) to obtain hybrid covers in a 7:3 ratio by weight, thus obtaining a 10% solution. The 10% PCL with the addition of 1/10% TWEEN 80 surfactant (Merck KGaA) was used as the other fiber-forming solution. The layers were deposited on the surface of nitinol (Kellogg's Research Lab), i.e. a shape memory alloy that has been used in cardiosurgery for many years. The nonwovens were obtained via simultaneous spinning of the fibers from the two solutions with different process parameters.
The obtained nonwovens were macroscopically assessed, their microstructure was observed, and the wettability and roughness of the surface were tested.

Results and Discussion

The proper selection the electrospinning process parameters allowed to obtain layers in the form of microfibers be considered applicable in medicine for the heart muscle reconstruction. The fibers had the unimodal diameter thickness distribution. The obtained hybrid coatings were characterized by variable wettability depending on the fiber type which dominated in the selected area. The contact angle equalled 130° if the drop hit the point with majority of the fibers lacking the TWEEN addition, and its value dropped to a few degrees if the PCL-based fibers with the addition were predominant.

Conclusions

By using the polymer solutions based on polycaprolactone, the hybrid layers with different morphology and properties can be obtained. The variety of coatings is achieved by changing: the substrates composition, the process parameters and the design of the electrospray device. In the presented studies, only a narrow scope of the conducted works was presented, which allowed to obtain a coating on nitinol applicable in cardiosurgery. The hybrid structure, the fibrous form and the varied local properties can support the healing process and constitute the scaffolding for tissue reconstruction.

Acknowledgments

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WHEY PROTEIN ISOLATE HYDROGELS FOR TISSUE REGENERATION AND DRUG DELIVERY

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Introduction

Whey Protein Isolate (WPI) is an inexpensive byproduct from the dairy industry. WPI is used as a component of drinks and as a protein supplement for bodybuilding. Addition of WPI to bone-forming cells has led to improved proliferation and osteogenic differentiation in previous work [1]. The main component of WPI is the globular protein beta-lactoglobulin (β-LG) which undergoes denaturation upon heating. This facilitates formation of crosslinks between β-LG molecules. Thus, WPI solution can heated to form a crosslinked network, resulting in formation of hydrogels whose Young's modulus are similar to 1 MPa. These hydrogels support the adhesion and proliferation of several cell types which relevant for bone regeneration [2-3]. Importantly, such hydrogels can be sterilized by autoclaving, which is an important practical advantage for clinical applications.

WPI hydrogels show potential in drug-delivery. During the gelation process, hydrophobic regions of β-LG are exposed, which not only enable formation of bonds and crosslinking of β-LG molecules due to hydrophobic interactions, but also present a large area within the hydrogel for the binding of hydrophobic molecules. Hence, hydrophobic drugs which are almost insoluble in water can be solubilized within the hydrogel. For example, the small hydrophobic molecule phloroglucinol (PG) can be solubilized in hydrogels to concentrations of over 5% (w/v) (FIG. 1). The use of hydrophobic drugs also allows a slow release, which is beneficial in many applications, especially when fighting microbial infections. The hydrogels have acted as delivery vehicles for poorly water-soluble biologically active substances such as polyphenols and small hydrophobic molecules with antibacterial activity like PG [3-4]. WPI hydrogels can also be combined with a mineral phase to form composite materials [5]. This talk will give an overview of our work with WPI hitherto.

Materials and Methods

WPI (BiPro) (Agropure, USA) solutions with concentrations of 15% (w/v) and higher form hydrogels when heated to 90ºC, which can then be sterilized by autoclaving at 121ºC and 1.1 bar. Particles or molecules can be incorporated into hydrogels during hydrogel formation. At 90ºC, hydrogel formation occurs within 5 minutes, which hinders sedimentation of particles. Antimicrobial tests are described in detail in [3].

Results and Discussion

PG, which is poorly soluble in water, can be solubilized in hydrogels to concentrations of over 5% (w/v) (FIG. 1) [3]. PG retains biological activity after sterilization by autoclaving and kills a range of microbes which occur in healthcare-associated infections (HAI), such as *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginos*a [3] (FIG. 2).

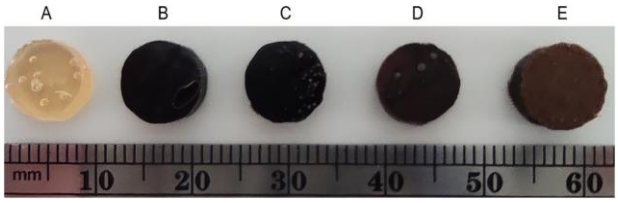

FIG. 1. Macroscopic images of disc-shaped WPI samples containing Phloroglucinol (PG) prepared from hydrogels solidified and autoclaved in petri dishes. WPI concentration is 40% (w/v), PG concentration ranges from 0% to 20% (w/v). A: WPI-PG-0%, B: WPI-PG-2.5%, C: WPI-PG-5%, D: WPI-PG-10%, E: WPI-PG-20%. Taken from [3].

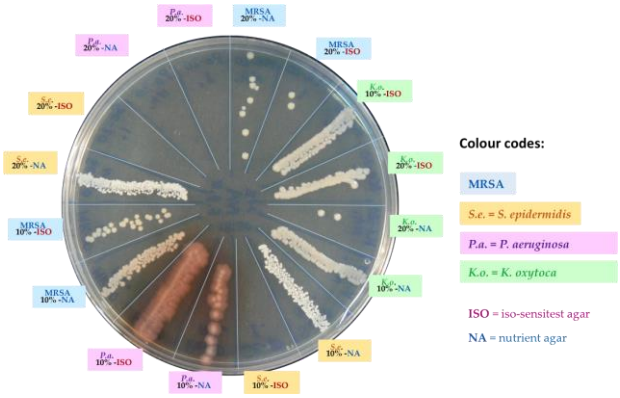

FIG. 2. Testing modes of growth inhibition after exposure of bacteria to disc-shaped hydrogels containing 10% and 20% of phloroglucinol. After sensitivity tests on Iso-sensitest (ISO) and nutrient agar (NA), areas of ZOI without bacterial growth were touched and streaked out onto sectors of nutrient agar plates and incubated overnight at 37°C. The absence of growth indicates bactericidal inhibition; abundant growth results from bacteriostatic inhibition; reduced number of colonies implies combination of bactericidal and bacteriostatic effects. Taken from [3].

Similarly, different tannic acids, which are poorly soluble in water, can be incorporated into hydrogels [4]. In other work, particles of hydroxyapatite (HA) have been incorporated into hydrogels containing WPI and influenced the osteogenic differentiation of behaviour adipose tissue-derived mesenchymal stem cells positively [2].

Conclusions

WPI is an inexpensive biomaterial from which hydrogels can be made easily. Incorporation of hydrophobic biologically active molecules and/or ceramic particles is straightforward. Biologically active molecules such as PG retain their biological activity after sterilization.

Acknowledgments

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REINFORCED COLLAGEN GEL WITH ADIPOSE TISSUE–DERIVED STEM CELLS AND ENDOTHELIAL CELLS FOR CARDIOVASCULAR TISSUE PATCHES

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Introduction

Type I collagen is a major extracellular matrix (ECM) protein that can be used as a 3D scaffold for soft tissue replacements, e.g. cardiovascular patches [1]. Adipose tissue-derived stem cells (ASCs) are able to differentiate into smooth muscle cells. Their growth and differentiation can be controlled experimentally by various stimuli, e.g. growth factors, scaffold stiffness, gel reinforcement, cell seeding density, co-culture with other cells, mechanical loading, etc. [2,3]. The study aimed to prepare reinforced collagen gels with ASCs and endothelial cells, and to evaluate the growth and differentiation of ASCs using various culture conditions.

Materials and Methods

Type I collagen was isolated from porcine skin and processed into a gel (final concentration in samples of 3 mg/ml). Collagen fibers (based on type I collagen, VUP Medical, Czech Republic) were prepared via electrospinning (4SPIN, Contipro, Czech Republic), crosslinked in ethanol with EDC+NHS, washed, frozen, and lyophilized. In order to prepare collagen particles, collagen fibers were swollen in ethanol, homogenized, washed, frozen, and lyophilized. They formed nano/submicron-scale particles; their concentration in collagen gel was 20 mg/ml. Polyvinyl alcohol (PVA) nanofibers and PVA nanofibers with platelet lysate (PL) inside (PVA_PL) were prepared via electrospinning. PL was isolated from donors blood, Liberec Hospital, Liberec (Liberec Regional Hospital, Liberec). Human ASCs were isolated from lipoaspirates obtained by liposuction (Bulovka Hospital in Prague). The ASC suspension was admixed into collagen solution (in acetic acid) with particles (final cell density of 500,000 cells/ml), and sodium bicarbonate was added. Collagen fibers were placed on the top of the formed gel. Collagen gels/composites with ASCs were cultured in EGM-2 medium (Promocell) or in DMEM with 2% with/without PVA or PVA_PL nanofibers or with TGFβ1+BMP4 for 7 and 14 days. The samples were endothelialized using human umbilical vein endothelial cells (HUVECs). On days 7 and 14, the gels were stained by immunofluorescence for alpha-actin and calponin and scanned by LightSheet, Zeiss and Bruker Ultima IntraVital microscopes. The cell densities were analyzed using Imaris image analysis software. Biomechanical evaluation was performed using a compression test (MTS Mini Bionix 858.02 system, USA). Mass spectrometry of endothelialized samples was performed after 12 days of culture. Statistical evaluation was performed using One Way ANOVA analysis.

Results and Discussion

Gel reinforcement with collagen particles or/and fibers reduced the gel shrinkage, allowed growth of ASCs, and supported their differentiation towards smooth muscle cells, which was manifested by more intense staining of calponin (FIG. 1). Similar positive effect on ASCs growth and differentiation occurred after supplementation of the cell culture medium with either PVA_PL nanofibers or with TGFβ1+BMP4 growth factors. However, the latter additive caused strong gel shrinkage and inhibited endothelial cell growth. Young modulus of all gels reinforced with particles increased in time. Both cell types in the composites with collagen particles produced ECM proteins and remodeling proteins. Reinforcement of the gels, growth factors or their slow release from nanofibers are useful tools for improving graft properties [3].

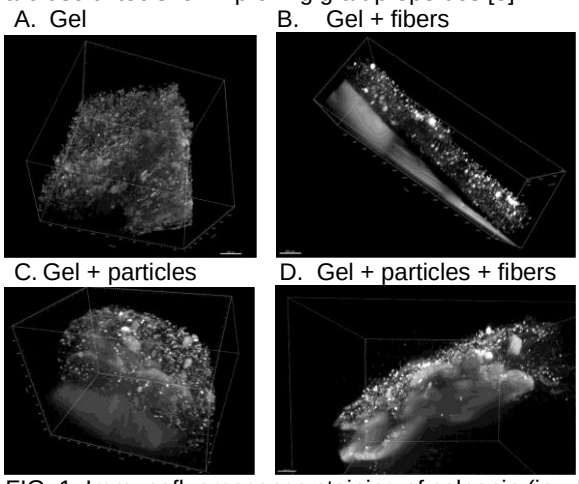

FIG. 1. Immunofluorescence staining of calponin (in white color) in ASCs embedded in collagen gel with various reinforcement: collagen fibers and/or collagen nano/submicron particles on day 14 of culture, EGM-2 medium, LightSheet, Zeiss, obj. 20x, tile scan.

Conclusions

Reinforced collagen gels supported ASC differentiation towards smooth muscle cells and collagen remodeling. The reinforcement reduced the sample shrinkage and improved mechanical manipulation, which is essential for potential usage as vascular patches.

Acknowledgments

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STEM CELL-BASED THREE-DIMENSIONAL CONSTRUCTS FOR FAT GRAFTS

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Introduction

There is an increasing need for appropriate replacements of soft tissues irreversibly damaged or missing after accidents, in case of developmental disorders, or after surgical ablations, e.g. due to tumours. Autologous fat grafts, commonly used for tissue augmentation, e.g. after breast cancer surgery, are prone to postoperative atrophy and fat absorption [1]. A promising approach to construction of more durable tissue replacements is the use of cell-material constructs containing autologous adult mesenchymal stem cells [2] seeded on three-dimensional (3D) nanofibrous scaffolds mimicking the architecture of the natural extracellular matrix [3]. The final aim is to vascularize these replacements, which still remains a challenge even in advanced tissue engineering.

Materials and Methods

In our work, we tested the cotton wool-like porous 3D scaffolds made by centrifugal spinning using NANOCENT equipment (DHB Technologies), prepared from bioresorbable polymers such as polylactide (PLA), polycaprolactone (PCL), polyhydroxybutyrate (PHB), poly(1,4)-butylene succinate (PBS) and their copolymers. Scaffolds were characterized by scanning electron microscopy (SEM), and by Brunauer–Emmett–Teller (BET) analysis of the pore size and of the specific surface area of the scaffolds. As a cellular component, human adipose tissue-derived stem cells (ASCs) and human umbilical vein endothelial cells (HUVECs) were used. ASCs were seeded onto the prepared scaffolds and were cultured for 10 days. The cell adhesion, number, morphology and penetration inside the scaffolds were followed by fluorescence staining with TRITC-conjugated phalloidin and DAPI. The cell adhesion, number and morphology was observed after fixation with 4% paraformaldehyde and using an epifluorescence Olympus IX71 microscope, while the cell penetration inside the scaffolds was followed mostly on unfixed cells using a Dragonfly 503 confocal microscope. The cell viability was tested by a resazurin metabolic assay. The possibility of vascularization of the scaffolds was tested in experiments with co-cultivation of ASCs and HUVECs. The endothelial cells were then distinguished by immunostaining of specific endothelial markers, namely E-cadherin and CD31 (PECAM-1). Simultaneously we attempted to differentiate ASCs towards pericytes and adipocytes. The differentiation of ASCs for purposes of adipose tissue development was performed for 12 days in an adipogenic medium based on DMEM containing 10% fetal bovine serum, insulin (10 µg/mL), dexamethasone (1 µM), and indomethacin (20 µM). Adipogenic medium supplemented with isobutylmethylxanthine (0.5 µM) or adipogenic medium containing rabbit serum was also tested. The adipogenic differentiation was estimated by Oil Red O staining and followed by Olympus IX71 microscope.

Results and Discussion

We have found that the most appropriate cotton wool-like scaffold is the PLA/PCL copolymer with statistically oriented fibers (i.e. obeying a probability distribution of orientation) and with sufficiently big pores (50-100 µm in diameter). The cells were spread along the fibers, proliferated well, and colonized subsequently the inside of the scaffolds to the depth of approx. 400 µm during 6 days of cultivation (FIG. 1). The ability of ASCs to penetrate inside the scaffolds is an important prerequisite for the successful creation of functional 3D tissue replacements. HUVECs also penetrated inside this scaffold sufficiently. The co-cultivation of ASCs with cells was most successful in of the ratio 1:1, detected endothelial structures could be the starting points for graft vascularization. As the optimal medium for ASC adipogenesis, the adipogenic medium containing rabbit serum was found.

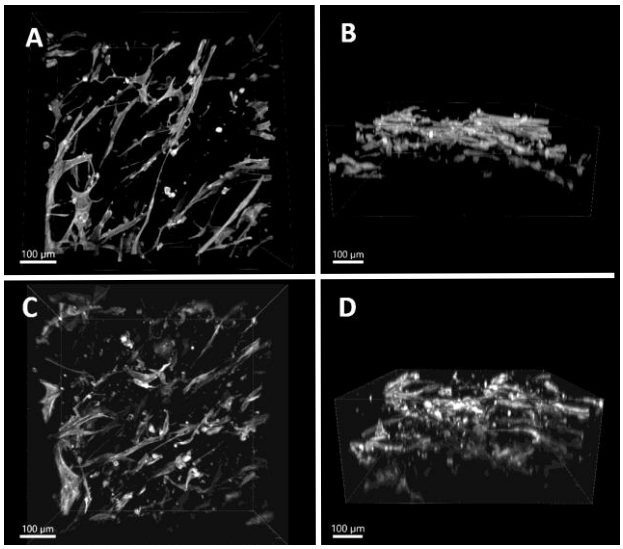

FIG. 1. The PLA/PCL scaffold seeded with ASCs after 2 days (A, B) and 6 days (C, D) of cultivation in DMEM. Cells were visualized by phalloidin staining (*in white color*). Top view (A, C), side view (B, D); Dragonfly 503 confocal microscope (obj. 20x), scale bar 100 µm.

Conclusions

PLA/PCL in the form of 3D fibrous scaffolds seems to be the most suitable material for creating a stem cell-based fat graft. It enables a sufficient penetration of ASCs inside the construct and simultaneous co-cultivation of ASCs with endothelial cells in order to promote the vascularization of the construct.

Acknowledgments

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GUIDED BONE REGENERATION BY PLANT-DERIVED NANOPARTICLES IN OSTEOPOROSIS

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Background
The repair and treatment of large bone defects in patients with compromised bone metabolism due to ageing and medical conditions such as osteoporosis present often a clinical challenge. Therefore adjunctive methods to enhance bone healing are needed. Bone tissue engineering with application of nanotechnology allows to construct biomaterials with desired properties being osteoconductive, osteoinductive and osteogenic.

Aim/Hypothesis
The aim of our study was to promote bone regeneration using functionalised scaffold with Rhamnogalacturonan-I pectins (RG-I) in vitro and in vivo using aging and osteoporotic rodent models.

Material and Methods
The biomaterials were poly(l-lactide-co-ε-caprolactone) scaffolds and the RG-I was from potato. The chemical and physical properties of functionalised biomaterials with RG-I nanoparticles were characterised using confocal and atomic force microscopy. Functionalised scaffolds with RG-I (tested sample) were evaluated in vitro with human osteoblasts from osteoporotic patients and their response was tested using real-time PCR. In vivo evaluation was performed using criticalsize calvaria bone defect model in ageing and osteoporotic rat models. Scaffolds were implanted randomly in the calvaria defects of aged female Wistar rats (11-12 months old) and osteoporotic female Wistar rats induced by ovariectomy. The control was scaffold without RG-I. After 2 and 8 weeks animals were euthanised. Harvested samples were analysed for osteogenic and inflammatory markers using real-time PCR. Bone formation was evaluated radiographically and histologically. The data was analysed using one-way ANOVA.

Results
The chemical and physical properties results indicated success of the functionalisation of scaffolds with RG-I. Osteoblasts response suggested osteogenic (upregulation osteopontin, osteocalcin, collagen1, bone sialoprotein) and anti-inflammatory properties (downregulation IL-1, IL-8, TNFalpha) on the scaffold functionalised with RG-I. The in vivo results in aged and osteoporotic rat calvaria model of early (2 weeks) bone regeneration showed increase of osteogenic markers and decrease of proinflammatory markers and RANKL, compared to control. In osteoporotic rat model at week 2 and 8 and in aged rat model at week 8, the mean percentage of BV/TV (bone volume/tissue volume) in the defect with RG-I scaffold was significantly greater than the defect with control. The histological evaluation in both rat models revealed larger areas of new bone formation in RG-I scaffolds than in control.

Conclusion and Clinical implications
In conclusion, the plant-derived nanoparticles significantly increased osteogenic and decreased pro-inflammatory response in vitro and in vivo. These finding may have a crucial impact on bone repair process especially in elderly and osteoporotic patients.

LDI PROCESS WITH THE USE OF SILVER NANOSTRUCTURED SUBSTRATES IN ANALYSIS OF LOW MOLECULAR WEIGHT COMPOUNDS

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Introduction

Mass spectrometric techniques can provide data on the composition of a studied sample, utilizing both targeted and untargeted approaches to solve various research problems. Analysis of compounds in the low mass range has practical implications in many areas of research and industry. Laser desorption ionization techniques are utilized for the analysis of molecules in a low mass region using low sample volume, providing high sensitivity with low chemical background. The fabrication of substrates based on nanostructures to assist ionization with well-controlled morphology may improve LDI-MS efficiency for silver nanoparticles with plasmonic properties. In this presentation, we report an approach for the preparation of silver nanostructured substrates applied as laser desorption ionization (LDI) plates, using the chemical vapor deposition (CVD) technique [1].

Materials and Methods

For the synthesis of the LDI plates, stainless steel (H17) was cut to pieces 2.5 × 7.5 cm. The surfaces of the steel samples (substrates) were covered by the silver coating, consisting of densely packed silver nanoparticles and microparticles (AgPs). For this purpose, a chemical vapor deposition (CVD) technique was used. In our CVD experiments, $Ag_5(O_2CC_2F_5)_5(H_2O)_3$ has been used as a precursor. The substrate surface preparation for the CVD process consisted of washing in an ultrasonic bath with distilled water containing a non-ionic surfactant for degreasing for 45 min (twice). Then, the substrate was immersed in the acetone for 30 min, then distilled water for 10 min and, after drying in an Ar stream, it was placed in a CVD reactor. The morphology of created coatings was studied using a scanning electron microscope. The structure of the AgPs films was investigated using an energy-dispersive X-ray diffractometer with a copper monochromator and CuKα radiation (λ = 0.15418 nm). XRD patterns were collected in the 2Θ range 10-80°, step 0.02° and time 20 sec. The Sartorius MCA2.7S-2S00-M microbalance has been applied to determine the weight of the reference sample before and after the CVD process. The stainless steel (H17) reference samples of sizes 1 × 1 cm were placed in the CVD reactor together with the investigated sample to obtain similar deposition conditions. For the purposes of the MALDI experiments, Ag films were prepared in real time, and the storage time of samples (in a closed box, at room temperature and with limited access to light) was not longer than 2–3 days. The LDI-MS performance of the synthesized plates was evaluated by using stock solutions at concentration of 1 mg/mL and standard mixtures of various lipids. Stock solutions of adonitol, glucose, fructose, shikimic acid, methionine, serine, alanine and phenylalanine were prepared by dissolving a powder of each standard in water. Stock solutions of cholesterol, oleic acid, palmitic acid and PC were prepared by dissolving a powder of each standard in chloroform. The standard mixtures of the various lipids were sonicated for 5 min prior to spotting to the target plate to avoid precipitation of lipids during storage. Subsequently, 1 µL of the stock solution of each compound and standard mixture was spotted to the synthesized LDI plates. LDI-MS analysis of low molecular weight compounds was carried out in both positive and negative ion-reflectron modes with the utilization of laser power at 80% in the mass range of *m/z* 60–1500. Analysis was performed using an UltrafleXtreme II MALDI-TOF-MS apparatus equipped with a modified neodymium-doped yttrium aluminium garnet (Nd:YAG) laser operating at 355 nm and frequency 2 kHz. Mass calibration was performed using signals of silver using quadratic and cubic enhanced calibration methods individually for each spectrum. Theoretical *m/z* values of the analyzed compounds were calculated by using ChemCalc program.

Results and Discussion

Our main idea was to study the dependency between the size of the deposited silver particles (AgPs), the coatings' surface morphology and the LDI plates' sensitivity to various low molecular weight compounds. For this purpose, the plates were subjected to characterization using scanning electron microscopy (SEM) and X-ray diffraction (XRD) techniques. The use of different masses of solid Ag precursor and similar deposition conditions enabled controlling the surface morphology of the deposited layers as well as controlling the size of the AgPs deposited. The presented approach allows for the synthesis of LDI plates with tunable sensitivity for various classes of small biomolecules. The utilization of a chemical vapor deposition technique with various values of the mass of the precursor resulted in the formation of structures with sizes 50–330 nm and up to 1 µm with irregular shapes. Small biomolecules were detected at nanomolar concentrations, while lipids were detected at the picomolar level with a reduced chemical background.

Conclusions

Depending on the mass of used CVD precursor, the approach allowed the synthesis of LDI plates with tunable sensitivity for various low molecular weight compounds in both ion-positive and ion-negative modes. Reduced chemical background and sensitivity to small biomolecules of various classes (fatty acids, amino acids and water-soluble metabolites) at nanomolar and picomolar detection levels for lipids such as triacylglycerols, phosphatidylethanolamines and lyso-phosphatidylcholines represent an emerging perspective for applications of LDI-MS plates for the collection of molecular profiles and targeted analysis of low molecular weight compounds for various purposes.

Acknowledgments

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STUDY ON LIPIDOMIC PROFILES OF POTENTIAL STARTER CULTURES BY LDI APPROACHES

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Introduction

Probiotics have been around humankind since people started consuming fermented milk and food. Nevertheless, their beneficial effects remained unexplored until the last century. During this time, the meaning of the word has been changed several times; how-ever, it has always been equated with health benefits. Currently, the "gold standard" in the identification of microorganisms is the sequencing technique 16S rRNA, which is characterized by high sensitivity, reliability, and reproducibility. However, the main problem is the long time needed to obtain the result and the high cost of the analysis [1]. In recent years, more and more emphasis has been placed on the search for modern, reliable and, at the same time, fast methods of detecting microorganisms. For such methods the MALDI MS technique can be included, in which identification is carried out on the basis of profiling proteome and comparisons with commercially available databases [2], but in the case The identification of close related environmental bacteria species is often imprecise. Because, after proteins, lipids are the main functional and structural component of cells and play an important role in many cellular processes such as membrane formation, energy storage, and signalling cellularity, they can be useful biomarkers for identifying microbes in a similar way the way in which protein-based platforms are used.

Materials and Methods

The two selected strains were analyzed and cultured for 48 h at 37 °C in liquid medium (MRS broth). After centrifugation, the biomass was washed in 1 mL of 0.9% NaCl solution, then lipid extraction was performed using the Folch method. A total of 1.5 mL of a 2:1 (vol/vol) chloroform/methanol mixture was added to the biomass. The Eppendorf tubes were transferred to an ultrasonic bath and left for 15 min at room temperature. Then, 0.5 mL of 0.05 M NaCl was added to the suspension and vortexed for 10 min. In the next step, the suspensions were centrifuged for 15 min at 2415× g, the upper layer was collected in separate test tubes, 0.5 mL of 0.05 M NaCl solution was added, and the tubes were vortexed again. The two bottom layers were combined, and the solvent was evaporated using a vacuum centrifuge. The lipid samples were dissolved in chloroform and a volume of 0.5 µL was applied to the plates immediately before measurement. A plate covered with a layer of silver nanostructures applied to the surface of H17 steel by means of electrodeposition was selected for the measurements [2]. Prior to the synthesis of silver nanostructures, the plates were cleaned in an ultrasonic cleaner with acetone, methanol, and acetonitrile. The two plates were then placed facing each other in a 100 mL beaker and connected to a Consort EV202 bench power supply. Ninety milliliters of silver trifluoroacetate solution (98%; Trimen Chemicals, Łódź, Poland) was poured into a beaker. The solution was prepared by dissolving 9×10^{-5} moles of AgTFA in a mixture of 80% isopropanol and 20% acetonitrile. Electrodeposition was carried out for 15 min at a voltage of 10V. The negative electrode was then cleaned with cotton wool and washed three times in boiling acetonitrile and isopropanol. LDI TOF MS measurements were per-formed in the m/z 80–2000 range with an UltrafleXtreme II mass spectrometer (Bruker Dal-tonics, Bremen, Germany) equipped with a 355 nm laser with a frequency of 2 kHz. The number of laser shots was 2000 (4 × 500) per sample. The electrode voltages were 26.64 and 13.54 kV. At the first acceleration, the voltage was 25.08 kV, and for the second ion source it was 22.43 kV. The detector gain value for the reflectron was 30×. Cubic calibration was performed using silver cluster signals. An analysis of the data obtained was per-formed using the FlexAnalysis 3.3 software (Bruker Daltonics, Bremen, Germany) and mMass 5.5.0.

Results and Discussion

Lactic acid bacteria are known for their production of bioactive peptides produced from proteins by microbial proteolysis; thus, it is not surprising that they were present in cell extracts. The remaining signals on the spectra were mainly assigned to lipids be-longing to all classes, i.e., fatty acids, glycerolipids, glycerophospholipids, sphingolipids, and steroids. Fatty acids (FA) are one of the most dynamic components of bacterial cells. They are components of phospholipids and play a key role in maintaining the structure and function of the cell membrane. The glycerolipids identified in the spectra were triacylglycerols (TGs) and diacylglycerols, which are a reserve and energy material in bacterial cells. Glycerolipids also regulate the level of lipids in cell membranes and are precursors in the synthesis of phospholipids. Among the glycerophospholipids identified were phosphatidylglycerols (PGs), which are the main phospholipids among lactic bacteria; phosphatidylethanolamines (PEs), responsible for membrane architecture and bacterial mobility; phosphatidylinositols (PIs), playing a role in the dynamics of the bacterial membrane; phosphatidylcholine (PC), which plays a key role in symbiotic interactions and resistance to bacteria and heavy metals; and phosphatidylserine (PS), which is a precursor in the synthesis of PEs [3].

Conclusions

LDI methods based on silver nanoparticles have an established position in the analysis of compounds with low molecular weights, such as metabolites or lipids [41]; however, it is worth noting that none of these techniques have thus far been applied to the analysis of lipids from microorganisms.

Acknowledgments

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CHALLENGES IN CLINICAL APPLICATIONS OF 3D-BIOPRINTED BIONIC ORGANS

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Abstract
Recently, tissue engineering, including 3D-bioprinting acquires clinical significance and becomes an outstanding, potential method of customized treatment in reconstructive medicine and several chronic illnesses including chronic pancreatitis or type 1 diabetes.
However, are we ready to implement this solution in the clinic? When will it be possible to print organs for transplantation? There is no clear answer to this question yet. However, it is worth paying attention to how much has already been achieved in the development of this technology and what issues remain to be solved.
Until recently, one of the main milestones to be achieved was the most accurate mapping of the cellular microenvironment in bioprinting models. The production of bioinks of natural origin was crucial to the functionality of the bionic constructs. It is known now that the most advantageous for this purpose is the use of a decellularized extracellular matrix (dECM) combined with biopolymers that enable crosslinking of the constructs with visible light. The most commonly used biopolymers are: gelatine, hyaluronic acid or chitosan. The mentioned biopolymers that underwent a previous methacrylation process show high biocompatibility with cells used in the bioprinting process and allow to maintain a stable structure for 12 months due to their photo-curable properties. We currently have a wide portfolio of biomaterials and significantly greater knowledge of 3D bioprinting. However, to use bioprinting in clinical application several hurdles should be overcome of scientific, technological and administration origin.
Scientific issues include: (1) bioprinting of the vascular system - internal and external. The bioprinting of the vascular system in the bionic organ is not unobtainable. Polish team bioprinted in 2017 the first bionic pancreas model with a vascular system in the world. However, the challenge for scientists remains to create a dense vascular network throughout the organ. Furthermore, a bionic organ intended for clinical use must have stable external vessels which will connect the organ with the recipients own blood flow. (2) Another problem to overcome is the mechanical strength of 3D-bioprinted organs, which should allow holding the pressure which is observed within human arteries, both physiological and in pathological conditions >200 mmHg). (3) Another group of problems is technological issues. Besides the bioengineering problems, there is no environment and technology ready to use to maintain the bioprinted organ outside the body. The medical devices for the storage of the organ after bioprinting is still under development. These devices should allow to asses bioprinted organs functionality and allow them to maturate the endothelium in the organ.
The final point to consider is administrative and regulatory issues. There are two aspects to be distinguished here: (1) regulatory issues in different countries and (2) duration of clinical trials and the actual implementation of the results obtained.
The divergence of regulatory pathways observed across different countries does not help bring new biotechnology to clinical trials and get Market Authorization for further development. Differences between the EU under the CAT administration and US with FDA recommendations sometimes leads to the need of doubling the efforts to perform study which will meet the criteria for both markets.
Last issue is long term for study before the treatment enters clinic, which includes medical devices, biomaterials, proper use of the cells and SOP for technology will be ready to use. This significantly diminishes investors' interest in developing technology. Because of that behaviour, preclinical trials often stop before clinical application even if it had excellent results.

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Conflicts of Interest
Michal Wszoła is the co-founders of Polbionica Ltd.

MONODISPERSE AND HIGH-PURITY PEG-PPG-PEG TRIBLOCK COPOLYMERS FOR BIOMEDICAL APPLICATIONS

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Introduction

Purity and defined chemical composition of all biomaterial components are essential for developing clinically safe and reproducible biotherapeutics. Poloxamers are widely applied in the biomaterial field, including several FDA approved formulations. Commercially available triblock copolymers of PEG-PPG-PEG are polydisperse materials with broad molecular weight distribution. Often these materials exhibit different chemical composition from batch-to-batch and contain unreacted monomers and potentially other impurities (FIG. 1) [1,2].
In the last decade, there has been growing interest in developing monodisperse poly(ethylene) glycols for bio-related applications. This approach has been proven to increase bioavailability and therapeutic efficacy of PEGylated proteins and small molecular drugs compared to polydisperse alternatives. Additionally, utilizing monodisperse PEGs allows for the possibility to fine tune physiochemical properties of the final biomaterial [3]. However, little attention has been devoted towards developing a similar strategy to synthesize and functionalize high purity and defined length triblock copolymers of PEG-PPG-PEG.
The aim of this study is to synthesize and characterize a single-length and high-purity triblock copolymer PEG_{15}-PPG_6-PEG_{15}. This approach is a proof-of-concept for the development of a library of poloxamers with defined length for each block and high purity.

Materials and Methods

PPG_6, $bnPEG_{15}Ms$ (MW = 846.4 g/mol, Polypure) and NaOH were used for the synthesis. Single-length PPG_6 oligomer (M_W = 366.56 g/mol) was obtained through displacement chromatography [4].
PPG_6 and $bnPEG_{15}Ms$ were dried in toluene under vacuum. PPG_6 and $NaOH_{(aq)}$ were mixed. Afterwards $bnPEG_{15}Ms$ was added. Reaction was run under vacuum in 60°C for 24 h. Upon completion, the reaction mixture was purified using displacement chromatography [4] and high-purity $bnPEG_{15}$-PPG_6-$bnPEG_{15}$ was obtained. This was then subjected to Pd/C-catalyzed hydrogenative (1 atm) deprotection of benzyl groups giving the final product - PEG_{15}-PPG_6-PEG_{15}.
The progress of the reaction was followed by LC/MS *(Agilent 6100 Series Quadrupole LC/MS)*. The final product PEG_{15}-PPG_6-PEG_{15} was additionally characterized by LC with ELSD detection (*Agilent 1260 Infinity II Evaporative Light Scattering Detector*).

Results and Discussion

PEG_{15}-PPG_6-PEG_{15} was successfully prepared in a two-step reaction. LC/MS was used for investigating each step of the synthesis, purity assessment, and molecular weight determination. LC/ELSD data additionally confirmed high purity and monodisperse character of the obtained triblock copolymer.

Conclusions

This work demonstrates the possibility to obtain high purity poloxamers with a specific length of each polymer block and strictly defined molecular weight. The future perspectives of this work include developing a library of monodisperse PEG-PPG-PEG with varying lengths of the blocks and post-synthesis derivatization with functional groups.

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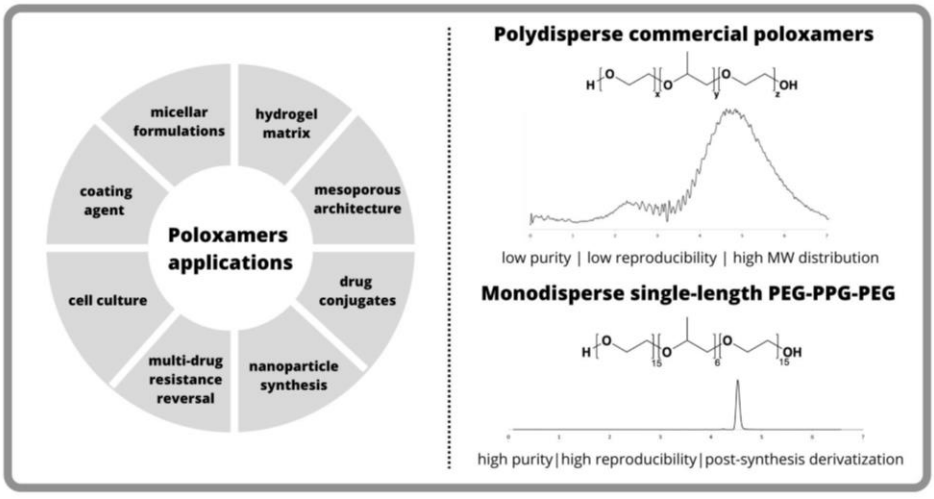

FIG. 1. Biomedical applications and comparison of polydisperse and monodisperse poloxamers.

INNOVATIVE MULTIFUNCTIONAL SYSTEM FOR LOCAL DELIVERY OF TMZ

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Introduction

Glioblastoma multiforme (GBM), the most malignant of the glial tumours is characterized by infiltrative character, high invasiveness, and a tendency to spread throughout the brain parenchyma. Although current therapies remain palliative, they have been proved to prolong overall survival [1]. The most extensively used chemotherapeutic is temozolomide (TMZ), since as one of few compounds that can cross the blood-brain barrier (BBB). However, the effectiveness of TMZ therapy applied orally or intravenously, is limited by the serious and dose-related side effects [2]. For the systemic toxicity of TMZ are responsible products of its rapid degradation, which are unable to exceed the BBB. Therefore, the solution that can address this issue would be the system for the local therapy, i.e., the material that could be implemented into the tumour bed just after its resection. To date there is only one clinically approved formulation of this type, releasing carmustine, however, no similar products containing TMZ are available. The proposed approach can increase TMZ concentration at the desired site simultaneously reducing adverse systemic complications [3]. Thus, the main objective of the proposed herein studies was the determination of the implantable material characteristics, further fabrication, and preliminary characterization of a system for local delivery of TMZ.

Materials and Methods

The lyophilized hydrogel-based material is composed of several differently serving elements that combined make the whole system multifunctional. Matrices of different compositions were tested to meet the demands of the implantable into the brain products [4,5].

The chemically crosslinked with a genipin three-dimensional network of biopolymers containing amino groups, namely: collagen/ lysine-modified hyaluronic acid (HAmod) and chitosan, acts as a falsework of the material. Built-in the polymeric matrix, ionically gelled with tripolyphosphate (TPP) chitosan particles, loaded with TMZ/vancomycin (VANC) are the carriers for therapeutic agents responsible for cytotoxicity against GBM and antibacterial properties, respectively. The series of materials were fabricated and characterized in terms of their physicochemical properties such as swelling, wettability, degradation as well as drug release experiments. To prove the possibility of a more tuneable TMZ release profile two modifications of the proposed system were applied. The former is related to surrounding the chitosan particles with HAmod layer, whereas the latter involves a transformation of TMZ into its derivative and further immobilization to biopolymeric chains constituting the hydrogel matrix. Synthesized carriers were characterized with regard to their morphology, size, and stability employing the scanning electron microscope (SEM), dynamic light scattering (DLS) technique, and zeta potential measurements, respectively. UV-Vis spectroscopy was applied for the estimation of the drugs' encapsulation efficiency. Synthesized TMZ-based derivatives were analyzed by Fourier-transform infrared (FTIR), and nuclear magnetic resonance (NMR) spectroscopies.

Results and Discussion

Based on the research on commercially available neurosurgeon devices/materials (TachoSil®/ Floseal®/ Clinisponge®) that are nowadays used during the resection we determined the features of the material for the local therapy and selected systems that meet them. It was demonstrated that by playing with materials composition their mechanical properties could be adjusted. Due to the application of the lyophilization process, the swelling ratio of the obtained systems was reduced when compared to the hydrogel materials and values of this parameter were closer to commercial materials. Complete wettability promotes adhesion to the tumour bed and slow mass reduction after materials' incubation in the artificial cerebrospinal fluid ensures stability at the site of implantation. SEM microphotographs revealed the porous nature of the system and confirmed that various types of particles are successfully embedded into the polymeric matrix. An increase in particles' size along with changes in the zeta potential value, from positive for chitosan particles to negative for carriers with an additional HAmod shell, demonstrated the successful functionalization. By means of UV-Vis measurements, the encapsulation efficiency of TMZ/VAN was estimated. The release profile study has shown that TMZ encapsulated in bare chitosan particles exhibited the burst release, therefore the need for further carriers' modifications was revealed. NMR and FTIR analyses identified the products of TMZ transformation and its next immobilization to biopolymeric chains.

Conclusions

The lyophilized hydrogel-based material exhibits a range of properties, which suggest that it can successfully serve as an implantable product. The presented herein formulation could pose a reasonable starting point for the development of a novel multifunctional system for local delivery of TMZ. The therapeutic effect as well as antibacterial features of fabricated materials, need to be verified *in vitro*.

Acknowledgments

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BIOCOMPATIBLE, BIOACTIVE, AND INJECTABLE HYBRID SYSTEM BASED ON HYDROGEL AND MESOPOROUS SILICA LOADED WITH ALENDRONATE FOR OSTEOPOROSIS TREATMENT

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Introduction

Osteoporosis is one of the most progressive, systemic, and metabolic diseases affecting bone tissue [1]. It is manifested by reduced bone mass and microarchitectural deterioration [2]. Alendronate (ALN), nitrogen-containing bisphosphonate, is the leading drug in the treatment of osteoporosis. ALN is characterized by limited oral absorption and bioavailability (0.9-1.8%) [3]. The need for the high dosage to be consumed can cause side effects mainly from gastrointestinal track associated with oral use (dyspepsia, esophagitis, vomiting, nausea, abdominal pain) [4]. Therefore, to improve bioavailability, limit the side effects from the ALN oral delivery, and enhance overall therapeutic potential, the novel injectable hybrid systems composed of hydrogel and inorganic ALN carrier (mesoporous silica functionalized with amino groups and decorated with hydroxyapatite) are proposed herein. Hydrogels, as a 3D matrix with pores of different size, is permeable for nutrients and oxygen and therefore can allow for cell growth. Additionally, they can integrate with surrounding tissue [5]. The selection of components for this matrix is mainly motivated by the composition of the extracellular matrix (ECM). Collagen is the main component of ECM as well as bones. Chondroitin sulfate is the representative of GAGs (glycosaminoglycans) that can stimulate the synthesis of collagen II, as well as bind calcium and calcium phosphates thus stimulating the local osteoblast adhesion. Chitosan is a linear polysaccharide that not only has antibacterial and antifungal properties but also accelerates wound healing [6]. The main disadvantage associated with hydrogels is their poor mechanical properties. One solution to overcome this problem is the incorporation of inorganic particles. Mesoporous silica (MSP) is a promising candidate for drug delivery due to its regular structure, biocompatibility, large surface area, and the possibility of functionalization of the inner and outer surfaces [7]. Hydroxyapatite (HAp) closely resembles the mineralized phase of bone and tooth. HAp is a bioactive material and is able to bind to the recipient's tissue after in vivo implantation. Additionally, it has a high affinity for alendronate [3].

Materials and Methods

The hybrid systems composed of 50wt% collagen, 20wt% chitosan, and 30wt% lysine functionalized chondroitin sulfate with 0.5mg/1.25mg/2.5mg of MSP-NH_2-HAp-ALN per 1 ml of sol (denoted as HybC1, HybC2, HybC3) were chemically crosslinked with genipin. The obtained polymeric sols were vortexed and placed in an incubator at 37°C until gel formation. Fabricated in mild conditions MSP-NH_2-HAp-ALN particles were characterized with complementary methods (FTIR, XPS, SEM, TGA, porosimetry). Degradability [collagenase solution type I (0.2 mg/ml, 1ml, ≥125 U/mg) in 1X PBS with 0.36 mM $CaCl_2$], swelling, biomineralization (SBF solution prepared with Kokubo's method), *in vitro* release of alendronate in PBS were assessed at 37°C with gentle shaking. Experiments were performed in triplicates and results are presented as averages. For the biological tests, the MG-63/J774A.1 cell lines were seeded onto the materials. After 1,3,7 day of culture, the cell viability was studied using Alamar Blue (AB) test. The alkaline phosphatase (ALP) activity of MG-63 cells was studied on the 3rd and 7th day of culture. Rheological evaluation and injectability were also performed.

Results and Discussion

The addition of particles into the hydrogel matrix caused a decrease in wettability and swellability and the effect was more pronounced in systems with higher particle concentration. The ability of hybrids to be used as injectable systems was established. The incorporation of MSP-NH_2-HAp-ALN into the hydrogel matrix improved its mechanical properties and the concentration-dependent effect was revealed. A bioactivity study demonstrated that the formation of new apatite-like inorganic structures is more pronounced for HybC2 and HybC3. Prolonged release (up to 20 days) and limited burst release effect make the hybrid system superior as the drug delivery system compared to pure MSP-NH_2-HAp-ALN particles. The biocompatibility of prepared systems was proven with Alamar Blue and ALP activity tests as the materials not only supported the proliferation of MG-63 cells but also their differentiation. Prepared hybrid systems also hindered the proliferation of model osteoclast cell lines, which in turn proved their therapeutic potential as the materials for the treatment of osteoporosis bones.

Conclusions

The aim of this work was to prepare hybrid systems composed of a hydrogel (Collagen:Chitosan:Modified chondroitin sulfate weight ratio 50:20:30 crosslinked with 20mM genipin solution) and functionalized with amine groups mesoporous silica particles, decorated with apatite and loaded with alendronate (MSP-NH_2-HAp-ALN) as a potential, injectable material for osteoporosis treatment.
It was found that fabricated injectable materials were not only biocompatible as they supported the MG-63 cell line proliferation and differentiation but also exhibited therapeutic potential as they hindered model osteoclast cell line growth, which makes them promising injectable and bioactive materials for small osteoporotic bone defects.

Acknowledgments

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INVESTIGATION OF HYALURONIC ACID PROPERTIES AS A SYNOVIAL FLUID SUBSTITUTE

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Introduction

The synovial joints play a very important role in the human body. They are responsible for proper mobility and load transfer. Hyaluronic acid, proteoglycans, and surface-active phospholipids are considered the basic biomolecules of synovial fluid that perform lubricating functions [1,2]. There is approximately 2 ml of synovial fluid in a healthy knee joint [3]. The synovial fluid is viscoelastic. It has sticky and elastic properties, and the dominance of one over the other is strictly dependent on the acting shear force. The viscous properties of the synovial fluid indicate the dissipation of mechanical energy, most often in the form of heat. On the other hand, elastic properties are responsible for the storage of mechanical energy [3,4]. The synovial joint is a biotibological system with a very complex friction system, where it is difficult to define a specific lubrication mechanism. Nevertheless, a very important function of synovial fluid is to keep the friction coefficient at a very low level [1,4]. Any changes in the chemical composition of the synovial fluid and any disturbances related to its proper functioning have a negative impact on the biofunctionality of the synovial joints. This can lead to damage to the articular cartilage and any diseases and inflammations of the entire joint [4].

Materials and Methods

The preparations were prepared with three different concentrations (4%, 6%, and 8%) of hyaluronic acid sodium salt with a molecular weight of 30,000-50,000 Da. Phosphate buffer (PBS) was used as a solvent. The prepared preparations were kept for 24 hours in an incubator set at 37°C. The proprietary compositions were compared with the available commercial product HYALGAN. From the basic physicochemical tests, the pH was measured. In the next stage, plate-to-plate rheological tests were performed using the Thermo Scientific HAAKE RheoStress 6000 rheometer. The oscillation tests as a function of frequency were performed. These measurements allowed us to determine how the modulus of G 'and G' 'changes as a function of the variable frequency f in the range from 0.1 to 10 Hz, with the constant value of deformation γ = 0.01. The friction tests were carried out on a Bruker UMT-1 tribometer, which worked in the sphere-disc system. The cylinder-shaped sample (8x5 mm) was made of CoCrMo cobalt alloy. The counter-sample made of alumina ceramics (Al_2O_3) had the shape of a sphere with a diameter of 6 mm. The friction tests were carried out at the frequency of 1 Hz, the motion amplitude of 500 μ, and the pressure force of 5 N, for 30 minutes. a felt base with the addition of a water suspension with aluminum oxide.

Results and Discussion

The pH values for the prepared compositions are in the range of 8.53-8.57. On the other hand, for the commercial preparation of Hyalgan, the value was 7.47. The pH of the natural synovial fluid is about 7.29-7.45.

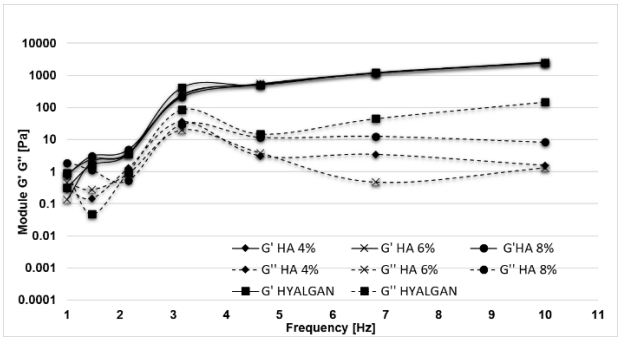

FIG. 1. The dependence of the module G 'I G' as a function of frequency.

In rheological studies, in all tested preparations, the modulus of elasticity G '(marked with a solid line) is usually higher than the modulus of viscosity G' '(marked with a broken line) (FIG. 1). Namely, as the shear rate (frequency) increases, the solutions become more elastic.

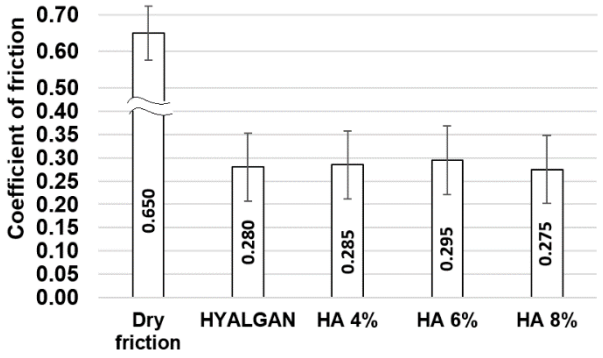

FIG. 2. Value of the coefficient of friction at the 30th minute of wear or rubbing.

The tribological tests show that the highest resistance to the motion was achieved in the conditions of dry friction (FIG. 2). On the other hand, all tested compositions reduce the friction coefficient. Own compositions show a similar course to the commercial preparation HYALGAN.

Conclusions

The viscoelastic nature of the liquid depends on the rate of deformation during deformation. This behavior of the liquid can result in the formation of a support layer on the articular surfaces. The obtained data show that all tested substitutes for synovial fluid have a positive effect on the reduction of resistance to motion in the friction node. The most favorable effects were obtained with the hyaluronic acid solution with a concentration of 8%.

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FISH COLLAGEN AND CHITOSAN MIXTURES AS AN PROMISING BIOMATERIAL FOR POTENTIAL USE IN THE MEDICINE AND THE COSMETIC INDUSTRY

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Introduction

Collagen is a structural protein and varies into 29 genetically different types. This biopolymer is mostly obtained from mammalian tissues, but due to the risk of carrying specific zoonotic diseases, collagen obtained from fish wastes became a safer alternative [1].
Even though collagen is an excellent biomaterial, it exhibits some limitations which can be conquered by mixing it with another biopolymer, such as chitosan.
Chitosan is a polycationic polysaccharide produced by the chitin deacetylation process. Just as collagen, chitosan can be formed in many different forms and has valuable and demanded properties, such as biocompatibility, biodegradability, and wound healing [2]. When added to collagen, chitosan can enhance the quality of the mixture due to its excellent mechanical and antimicrobial properties [3]. Such mixtures can be used as functional biomaterials in the medical, pharmaceutical, and cosmetic industries [1].
This study aimed to obtain thin films from mixtures of low and medium molecular weight chitosan with fish collagen in three different ratios and examine their features for potential use in medicine and cosmetic industry.

Materials and Methods

Collagen from skins of Silver carp (Hypophthalmichthys molitrix) was purchased from WellU sp. z o.o., Gdynia, Poland. Low molecular weight chitosan (LCh) and medium molecular weight chitosan (MCh) was purchased from Sigma-Aldrich.
To obtain 2% solutions of collagen, and both kind of chitosan, each biopolymer was dissolved in 0.5M acetic acid.
Mixtures of previously prepared solutions were made by combining them in three different ratios: 25:75, 50:50, and 75:25.
Thin films were obtained by pouring 25 g of each solution onto the plastic plates with dimensions 100x100x20 mm.
To search the properties of mentioned films, the following equipment was used: Thermo Fisher SCIENTIFIC PIKE GladiATR NICOLET iS10, Waltham, MA, USA for obtaining IR spectra; Zwick/Roell Z 0.5 testing machine, Ulm, Germany for mechanical properties analysis; Goniometer with a system of drop shape analysis (DSA 10, Krüss, Germany for contact angle measurement; Multimode scanning probe microscope with a Nanoscope IIIa controller (Veeco Digital Instruments, Santa Barbara, CA, USA) for topographic imaging.
Swelling test of the films in PBS buffer solution with a pH of 7,4 was done. Statistical appraisal of the results was conducted with Q-Dixon's test.

Results and Discussion

Infrared spectroscopy analysis showed that in IR spectra of examined biomaterials, there are shifts in bands positions which show that intermolecular interactions between collagen and chitosan occur in the blends.

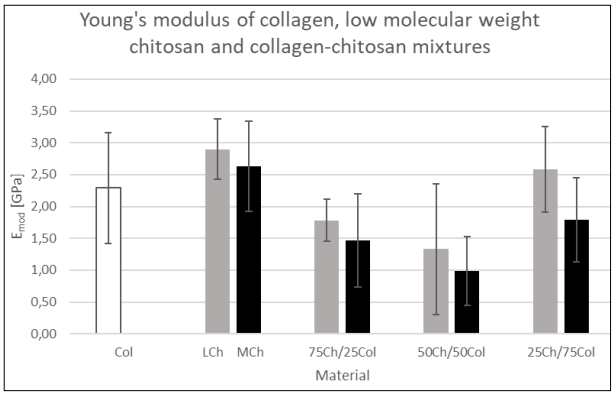

FIG. 1. Young's modulus (E_{mod}) of films made of fish collagen (Col - white), low molecular weight chitosan (LCh - gray), medium molecular weight chitosan (MCh – black) and mixtures of LCh and MCh (gray and black, respectively) with collagen in following ratios: 75:25, 50:50, 25:75.

The mechanical properties in the blends were different than those in single biopolymer film. Data presented in FIG. 1 demonstrates that chitosan films are more elastic than collagen and that film made of LCh is more elastic than the one made of MCh. In both cases, films obtained from 50:50 mixtures have got the lowest Young modulus and the ones from 25:75 mixtures have got the highest one.
Hydrophilicity and polarity of the blends decrease with increasing collagen content and this may suggest that the adhesion to the skin will be enhanced, because in general the skin surface is hydrophobic.
The topography of the surface of obtained films varies depending on the ratio of biopolymers in the mixtures.
Swelling tests indicated that chitosan absorbs more water than collagen. After 1 hour of soaking in PBS solution, almost all samples have fallen apart. Only a few made of collagen and blends with 75% collagen content remained integral for up to 4 hours.
Within the last three decades, an increasing interest in new materials based on blends of two or more polymers has been observed, so the data presented in this paper can be significant in the new materials development.

Conclusions

Properties of films made of collagen-chitosan mixtures vary depending on the molecular weight of chitosan and the content of each biopolymer in the blend.

Acknowledgments

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CURCUMIN-LOADED CHITOSAN-COLLAGEN ELECTROSPUN WOUND HEALING PATCHES

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Introduction

Dressing materials play a crucial role in wound healing processes being able to provide surface protection and drug delivery, regulate bacteriostatic activities, as well as accelerate cell and tissue regeneration. In this regard, electrospun patches based on naturally derived polymers (e.g., polysaccharides and proteins) possess specific properties that make them ideally suited for a wide range of biomedical and pharmaceutical applications [1]. Among others, the intrinsic biocompatibility and the unique nanofibrous structure of such materials provide them with a marked capability to foster cell viability, which can be further increased by using bio-active substances (e.g., antioxidants, anti-inflammatories, etc.) [2-4].
However, natural bio-polymers are commonly difficult to electrospun and require the employment of synthetic co-spinning agents and post-production treatments that might reduce their wound healing efficiency.
With these premises, we report here a robust protocol for the fabrication of curcumin-loaded composite electrospun patches comprised of chitosan and fish-derived collagen.

Materials and Methods

Nanofibrous mats were obtained via the electrospinning of chitosan, collagen, or chitosan-collagen solutions containing curcumin. Poly(ethylene oxide) (PEO) and Triton X-100 were used as co-spinnign agent and surfactant, respectively. Polymer and curcumin powders were solubilized in a 10 %v/v acetic acid solution under stirring for 24 h. Triton X-100 (1% wv) was then added and the mixtures were left under agitation for further 24 h before being electrospun. Temperature was maintained at T = 10°C for the entire solubilization process. In a typical electrospinning experiment, 20 mL of solution were electrospun on an aluminum drum collector. Processing parameters (voltage, spinneret-collector distance, flow rate) were varied depending on the working solution, whereas temperature and relative humidity were controlled at 20°C and 30%, respectively.

Results and Discussion

TABLE 1. Composition of solutions. Polymer concentration is expressed as % wv on the total volume, curcumin concentration as % wt on the polymer.

Mat	Coll	Chit	PEO	Curc
Coll	1.25	-	1.25	-
Chit	-	1.25	1.25	-
Coll+Chit	0.625	0.625	1.25	-
Coll+Curc	1.25	-	1.25	1
Chit+Curc	-	1.25	1.25	1
Coll+Chit+Curc	0.625	0.625	1.25	1

Once prepared, the mats were treated with NH_4 vapors for 4 h and then subjected to a 10 min irradiation with UV light. Such a protocol was specifically developed to prevent the mat dissolution in physiological conditions.
Such mats are characterized by a well-defined and homogenous nanofibrous structure (FIG. 1), high porosity, stability in a physiological-like environment, and adequate mechanical and water-related properties. Additionally, the presence of curcumin not only resulted in remarkable antioxidants properties but also seemed to promote cell adhesion and proliferation (FIG. 2).

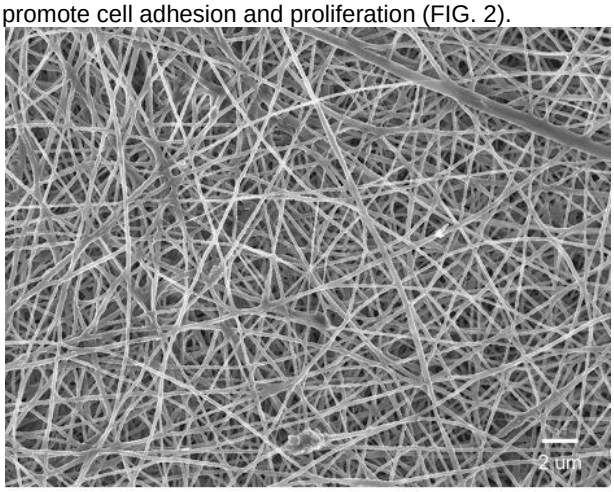

FIG. 2. SEM micrograph of chitosan-collagen electrospun mats loaded with curcumin.

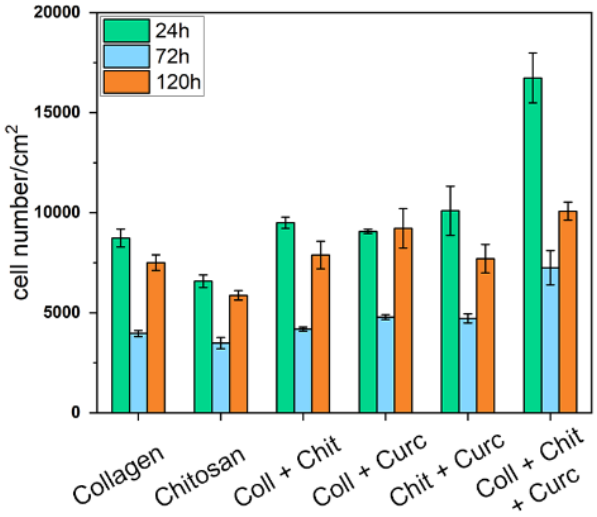

FIG. 2. Cell adhesion and proliferation on different electrospun mats.

Conclusions

In conclusion, the prepared electrospun patches represent a promising class of materials for applications in tissue engineering, wound healing and drug delivery.

Acknowledgments

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SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES SURFACE-MODIFIED WITH SELECTED POLYMERS FOR TARGETED ANTI-CANCER THERAPY

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Introduction

Up to 90% cases of cancer-related death are due to process called metastasis, in which tumor cells (CTC) detach from the main tumor side. They start to circulate in the bloodstream and travel to other organs, where they form secondary tumor. One of the materials which can be used to capture CTC are superparamagnetic iron oxide nanoparticles (SPIONs). They are small crystals of iron oxide, usually magnetite (Fe_3O_4) or its oxidised form, maghemite (γ-Fe_2O_3). The surface of SPIONs can be easily coated, stabilised and modified, offering us many different ways, in which we can adapt our nanoparticulate system to the needs of the application [1].
Our aim was to obtain SPION-based systems for effective and selective capture of CTC. SPIONs produced via co-precipitation method were stabilized with succinyl derivative of chitosan (SPION/NSCh) or alternatively with polyethyleneimine (PEI)/hyaluronic acid (SPION/PEI/HA) coating [2,3]. SPION/NSCh were further decorated with selected antibodies. We have also synthetized ultra-small SPIONs stabilized with oleic acid (SPION-OA) using thermal decomposition method [4]. These ultra-small SPIONs could further be loaded into the short, surface-modified halloysite nanotubes to obtain an alternative CTC capture system. All proposed systems may be used to target and selectively bind CTC.

Materials and Methods

Chitosan 85/100 (Heppe medical chitosan GmbH, Germany); Succinic anhydride, ≥99% (Merck, Poland); Iron (III) chloride hexahydrate, ≥99% (Merck, Poland); Iron (II) chloride tetrahydrate (Sigma-Aldrich, Poland); halloysite (HNT) nanoclay, medium size: 30-70 nm × 1-3 μm) (Sigma-Aldrich, Poland); Tetradecylphosphonic acid (TDP), ≥98% (Merck, Poland).
FTIR spectra were obtained with Nicolet IS10 spectrometer (Thermo Fischer) with ATR accessory. ^{1}H NMR spectra were measured using Bruker BioSpin GmbH. Hydrodynamic diameter and zeta potential were determined with Zetasizer Nano-ZS, Malvern. Thermogravimetric analysis was perfomed on 851e TGA/SDTA microthermogravimeter equipped with QMS Thermostar GSD 300 T Balzers (Mettler Toledo). Magnetic properties were determined using Vibrating Sample Magnetometer and 57Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer with 50 mCi 57Co/Rh source. MTT assay was performed according to procedure described in supplementary materials of publication K. Karnas et al. [1].
The NSCh was obtained in the reaction between chitosan and succinyl anhydride. SPION stabilized with NSCh (SPION/NSCh) and PEI//HA coating (SPION/PEI/HA) were obtained via co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of NSCh; in the case of the latter, a higher temperature (80ºC) was used. SPION/NSCh were then tosylated in reaction with p-toluenesulfonyl chloride and binded with selected antibodies. SPION-OA was synthesized by heating to 250ºC a solution consisting of oleic acid, oleylamine and 1,2-hexadecanediol with subsequent purification by repeatedly centrifuging the sediment and washing with n-hexane. Halloysite nanotubes (HNT) lumen were etched in reaction with acetic acid, then nanoclay was homogenised using sonication. Finally, earlier prepared HNTs were modified with tetradecylphosphonic acid (TDP). SPION-OA were then loaded under vacuum inside HNTs lumen.

Results and Discussion

NSCh was successfully synthetized and characterised, and its substitution degree was optimised. It was then used to obtain SPION/NSCh. The average size of the nanoparticles obtained was 136 nm ± 39, and their zeta potential was high (-34.2 ± 4.5 mV), confirming they were colloidally stable. The surface of SPION/NSCh was successfully decorated with antibodies. Magnetic measurements confirmed superparamagnetic character of the studied SPION systems. MTT assay showed that SPION/NSCh system is only slightly cytotoxic to HT-29 colon cancer cells after 24 h of incubation. The SPION/PEI synthesis was effective and the particles obtained had a size of 47 nm ± 15.6 and a zeta potential of +42 mV ± 4.1. The introduction of HA influenced both the size and the surface charge of the particles.
SPION-OA were successfully prepared and their size was ca. 7.0 nm ± 2.15. The studies of the magnetic properties of SPION-OA confirmed their superparamagnetic character. Halloysite nanotubes were effectively etched and their size was decreased and made uniform. No damaging consequences to the structure of the nanotubes were observed. Successful modification of their surfaces with TDP was confirmed by thermogravimetry measurements. Also, loading of SPION-OA inside modified HNTs has been studied.

Conclusions

We have synthetized and optimised different systems based on SPION nanoparticles designed for CTC capture. The physicochemical, magnetic and biological properties of the obtained systems were studied.

Acknowledgments

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EFFECT OF ZINC IONS INCORPORATED WITHIN THE STRUCTURE OF BONE SCAFFOLD ON CELLULAR RESPONSE AND ANTIBACTERIAL ACTIVITY *IN VITRO*

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Introduction

Modification of biomaterials with metal ions to improve their biocompatibility is a growing trend in the bone tissue engineering. One of the most current methods to incorporate ions in the structure of the biomaterials is the substitution of bioceramics with ions, such as magnesium (Mg^{2+}), zinc (Zn^{2+}), fluoride (F^-) [1]. The aim of this study was to synthesize nano-hydroxyapatite substituted with zinc ions (HA-Zn) that was used as a base for the fabrication of chitosan-agarose-hydroxyapatite (HA) scaffolds (chit/aga/HA and chit/aga/HA-Zn). The effect of incorporated zinc ions on cellular response *in vitro* (cytotoxicity, cell spreading, proliferation, and osteogenic differentiation) and antibacterial activity was evaluated.

Materials and Methods

HA and HA-Zn (content of Zn: x = 0.03 mol; 0.2 wt.%) nanopowders were synthesized by a wet precipitation method, as described earlier [2]. HA-based biomaterials (chit/aga/HA and chit/aga/HA-Zn) were fabricated in accordance with the procedure described in the Polish Patent no. 235822, by a combination of gas-foaming and freeze-drying methods. *In vitro* cell culture experiments were performed by using mouse calvarial preosteoblast cell line (MC3T3-E1 Subclone 4, ATCC-LGC standards) and human bone marrow-derived mesenchymal stem cells (BMDSCs, ATCC-LGC standards). Cytotoxicity evaluation of the biomaterials was performed by staining of MC3T3-E1 cells with a Live-Dead Double Staining Kit. Evaluation of cell spreading was carried out by seeding MC3T3-E1 cells directly on the scaffolds followed by fluorescent staining of cytoskeleton filaments and nucleus by AlexaFluor635-conjugated phallotoxin and DAPI, respectively. Then, spreading area of cells was measured using ImageJ software version 1.52a. Osteoblasts proliferation on the surface of produced biomaterials was evaluated by the total Lactate Dehydrogenase Activity Assay Kit. Effect of biomaterials on osteogenic differentiation of BMDSCs was assessed by quantitative analysis of typical osteogenic markers (type I collagen (Col I), bone alkaline phosphatase (bALP), osteocalcin (OC)) after 21-days culture by ELISAs kits. Antibacterial activity of biomaterials against *Staphylococcus aureus* and *Escherichia coli* was performed according to OECD Document No. 202 (JT03360420) by determining bacterial viability after direct contact with the scaffolds.

Results and Discussion

Cytotoxicity assessment of the biomaterials against MC3T3-E1 cells by live-dead staining showed that fabricated biomaterials were non-toxic (FIG. 1). Confocal laser scanning microscope images showed a great number of viable cells (green fluorescence) on the surface of chit/aga/HA and chit/aga/HA-Zn.

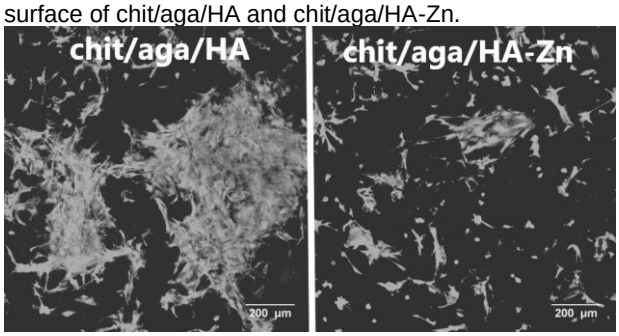

FIG. 1. Microscope images showing viable and well spread MC3T3-E1 cells on the scaffold surface [2].

As shown in a TABLE 1, the chit/aga/HA-Zn was slightly more favorable to cell spreading and proliferation than chit/aga/HA. In turn, evaluation of the level of typical osteogenic markers (Col I, bALP, and OC) produced by BMDSCs cultured on the surface of produced biomaterials showed that the addition of zinc to the biomaterial did not enhance synthesis of osteogenic markers as compared to the BMDSCs cultured on the scaffold made of pure HA. Thus, obtained results are not consistent with the reports in the available literature regarding the stimulative effect of zinc ions on the production of osteogenic markers by mesenchymal stem cells [3]. In this study, it was also proven that chit/aga/HA-Zn had strong antibacterial activity against *S. aureus* and *E. coli* (TABLE 1). Interestingly, material made of pure HA also showed antibacterial activity against *E. coli*.

TABLE 1. Biological response to chit/aga/HA and chit/aga/HA-Zn *in vitro* – the summary of obtained results.

Evaluation of cellular response to biomaterials			
Material	*Spreading area of MC3T3-E1 [µm²]*	*MC3T3-E1 cells proliferation [the amount of cells]*	*Osteogenic differentiation of BMDSCs [ng/mg]*
chit/aga/HA	2925 ± 1147	1-day: 1.08 x 10^4 6-day: 4.62 x 10^4	Col I: 36.31 bALP: 40.67 OC: 274.91
chit/aga/HA-Zn	3364 ± 1226	1-day: 1.24 x 10^4 6-day: 5.51 x 10^4	Col I: 26.43 bALP: 18.45 OC: 124.36

Antibacterial activity assessment [% CFU reduction]		
Material	*S. aureus*	*E. coli*
chit/aga/HA	46.80	92.74
chit/aga/HA-Zn	98.47	> 99.94

Conclusions

Obtained results showed that modification of chit/aga/HA scaffold with zinc ions does not significantly improve the biocompatibility of fabricated bone scaffold. Nevertheless, the developed biomaterial has excellent antibacterial properties without cytotoxicity, making it a promising biomaterial for biomedical applications.

Acknowledgments

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CURDLAN-BASED BIOMATERIAL ENRICHED WITH ZINC-DOPED HYDROXYAPATITE AS ANTIBACTERIAL DRESSING

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Introduction

The developing drug resistance among pathogenic bacteria is an increasingly common obstacle to the effective treatment of patients. This problem is generally noted in the treatment of infected wounds [1]. To counteract such a nuisance, the development of bioactive wound dressings is being pursued. For their production, both synthetic and natural polymers are increasingly used as a base. Such a matrix is widely enriched with additional bioactive components stimulating the healing process as well as having bacteriostatic or antimicrobial activity [2]. The aim of this work was to create a biomaterial based on a curdlan/agarose matrix [3] enriched with zinc-doped hydroxyapatite nanoparticles (marked as Mat HAP-Zn) for potential application as a wound dressing.

Materials and Methods

The curdlan/agarose matrix was prepared by mixing polymers in the water environment with preheating and mixing. After completely mixing the polymers the Zn-doped hydroxyapatite was added and mixed to obtain a white homogenous mass. Then the mixture was transferred to the vessel and incubated in a water bath at 95°C for 20 min. Subsequently, biomaterial was cooled to 4°C whereupon frozen (-80°C; 12 h) and subjected to a freeze-drying process for 16 h. The chemical composition of the biomaterial matrix (without HAP-Zn) and its production method was described in details in Polish Patent no. 236367, 2021, and was used as a reference sample and marked as Mat control (FIG. 1).
Human normal skin fibroblasts (BJ) obtained from ATCC were used as an *in-vitro* cellular model. The biocompatibility of the tested biomaterials was evaluated by indirect test (MTT) in accordance with ISO 10993-5 (2009). Cytotoxicity was analysed by preparing biomaterials extracts (24-h, 48-h and 72-h) from the tested dressing and then added to cells seeded to wells of the multiwell plate at a concentration of 1×10^4 cells. BJ cells were cultured for 24-h at 37°C, 5% CO_2 and then cytotoxicity was measured.
Antibacterial activity evaluation of developed biomaterials was assessed using direct contact test based on standard AATCC Test Method 100-2004 with modification. Following bacterial strains were used: *Staphylococcus aureus*, ATCC 25923 and *Pseudomonas aeruginosa*, ATCC 27853. Samples were infused with bacterial suspension and incubated at 37°C for 24 h. Then biomaterials were shaken to elute the bacterial cells next eluates plated onto Mueller-Hinton agar and incubated at 37°C for 48 h. Afterward, CFU number was counted and presented as a percent of the number of bacteria compared to the inoculated control specimen at T0.

Results and Discussion

The cytotoxicity assay presented that developed biomaterials were non-toxic to normal skin fibroblasts. However, the viability of cells upon contact with the 2 and 3-day extracts of biomaterials containing zinc-doped hydroxyapatite was reduced to approx. 71% compared to polystyrene control. It could have arisen by accumulation of higher concentration of zinc ions in 48-h and 72-h extracts compared to 24-hour extract (FIG. 2a).

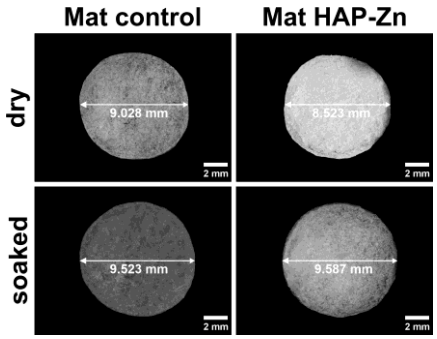

FIG. 1. Microstructure of produced biomaterials (scale bar = 2 mm).

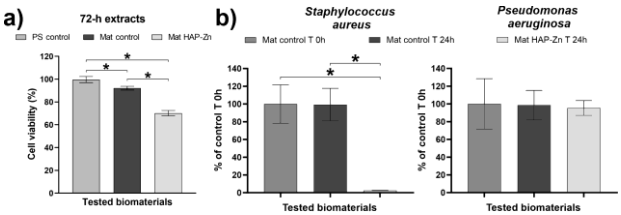

FIG. 2. a) MTT assay results for BJ cells cultured with 72-h fluid extract of the tested biomaterials, * statistically significant results compared to PS, b) antibacterial activity assessed by direct contact test (* statistically significant results compared to inoculated control material immediately after the inoculation – T 0h).

The antibacterial activity test exhibited that Mat HAP-Zn biomaterial reduced bacterial multiplication compared to the control biomaterial without antibacterial agent (FIG. 2b). Acquired results demonstrated a strong bactericidal activity about 99.9% reduction in the number of viable bacterial CFUs of Mat HAP-Zn against *S. aureus*. In case of *P. aeruginosa*, Zn-loaded material did not reveal antibacterial activity.

Conclusions

Dressing materials enriched with antibacterial metal ions are frequently applied to prevent bacterial infections during wound healing process. As part of this study, a biomaterial enriched with zinc-doped nano-hydroxyapatite was developed. Biomaterial revealed significant antibacterial activity against *S. aureus* and was non-toxic to human skin fibroblasts. Consequently, it may be potentially used as a wound dressing to reduce the risk of multiplication of bacteria during wound care.

Acknowledgments

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BIOCOMPATIBLE, NANO-COMPOSITE HYDROXYAPATITE/ POLYMER GRANULES FOR TREATMENT OF BONE DEFECTS

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Introduction

The most commonly used material in the treatment of bone defects is hydroxyapatite (HA). Sintered at high-temperatures hydroxyapatite is very popular and commercially available. This type of calcium phosphate is highly biocompatible, but also has a low specific surface area (SSA), which reduces its bioresorption and bioactivity. To obtain HA of high SSA, low sintering temperatures during HA production are used. Consequently, highly porous granules with a large SSA are formed. Its great drawback, however, is the toxicity to the cells [1-3]. Fabrication of composite biomaterials consisting of polymers and calcium phosphate ceramics seems to be a promising alternative to HA sintered at low temperatures. This kind of bioceramics is currently intensively studied as substitutes for bone tissue supporting its regeneration [4]. The aim of this research was to develop biocompatible, nanocomposite granules with high microporosity and a relatively high SSA for the treatment of bone defects. The nanocomposite material was created by combining nanohydroxyapatite (nanoHA) and two polymers of natural origin: chitosan and agarose.

Materials and Methods

The nanocomposite granules were prepared by mixing the appropriate amount of chitosan and agarose (marked as chit/agar/nanoHA) in acetic acid solution. Subsequently nanohydroxyapatite and sodium bicarbonate were added in the suitable proportions. The resulting paste in a special container was incubated in a water bath at 95°C, next the material was cooled, frozen at -80°C and freeze-dried. The obtained material was soaked in PBS solution and air-dried. The final step was granulating the material.

Cytotoxicity test was carried out using normal mouse calvarial preosteoblast (MC3T3-E1, ATCC) and normal human fetal osteoblast (hFOB 1.19, ATCC) cell lines. The cytotoxicity of the granules was assessed using the MTT test according to ISO 10993-5:2009 guidelines. The viability of the cells in direct contact with the material was evaluated by LIVE/DEAD staining and observation with CLSM. The degree of porosity of the biomaterial was investigated by the Mercury Intrusion Porosimetry (MIP) method, whereas the SSA was determined by the nitrogen adsorption technique using Brunaurer-Emmett-Teller (BET) theory. As reference materials low- and high-temperature sintered hydroxyapatite were used.

Results and Discussion

The MTT test demonstrated that the produced chit/aga/nanoHA granules were non-toxic to mouse preosteoblasts and human osteoblasts. Cell viability of both cell lines after 48 hours incubation with chit/aga/nanoHA extract was approximately 85% compared to the control (FIG. 1). The CLSM images showed viable, well-flattened cells with normal morphology, indicating good adhesion to the granules surface. Moreover, nanocomposite granules showed a high mesoporosity (47%, average pore diameter approx. 10 μm) comparable to low temperature sintered HA (51%) and a relatively high specific surface area (approx. 29 m²/g) (TABLE 1). The increase of these parameters compared to high temperature HA enhances the bioresorption capacity and bioactivity of the material [3].

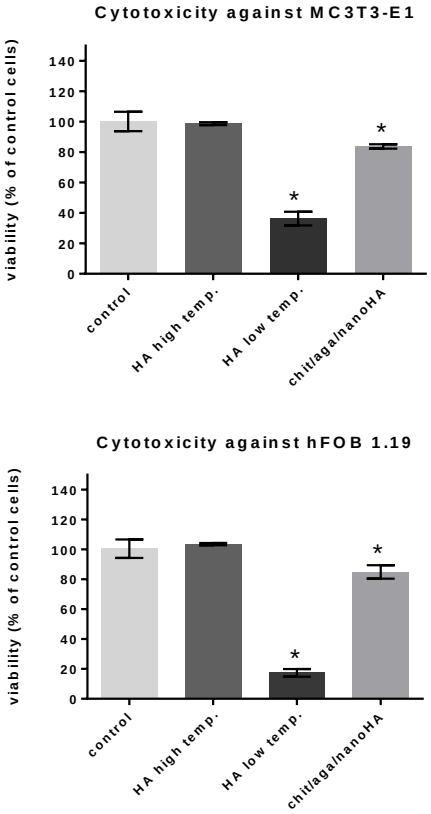

FIG. 1. MTT cytotoxicity test after 48 h of incubation (culture medium – control), $P < 0.05$, one-way ANOVA, (*statistically significant results compared to control).

TABLE 1. Porosity (%) and the SSA of the tested granules and HA sintered at low and high temperature.

Sample	Porosity [%]	SSA [m²/g]
HA high temp.	26,67	2,35
HA low temp.	51,04	58,32
chit/aga/nanoHA	47,01	28,65

Conclusions

The obtained results show that the chit/aga/nanoHA nanocomposite granules are characterized by high biocompatibility and improved microstructural properties. In addition, a large number of viable, properly flattened cells on their surface may indicate osteconductive properties. The produced hydroxyapatite/polymer granules may therefore be a promising alternative in the treatment of minor bone defects.

Acknowledgments

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THE INFLUENCE OF ALD SURFACE MODIFICATION OF Ti13Nb13Zr ON FUNCTIONAL PROPERTIES

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Introduction

Currently used titanium alloys, such as Ti6Al4V and Ti6Al7Nb, are being replaced by materials showing better biocompatibility. Replacing elements such as vanadium or niobium allows you to obtain the so-called new generation titanium alloys [1-3]. These alloys with the addition of Zr, Fe and Ta gain value approximately to the Young's modulus of bone, thus further improving the biocompatibility of the biomaterial. Modification of the surface of biomaterials plays an important role in determining the results of biological and material interactions. By appropriate modification, the surface of the material can be adjusted to improve biocompatibility, adhesion and cellular interactions. Therefore, surface modification is critical in the development and design of new biomaterials and medical devices. Surface modification of biomaterials provides an overview of both established surface modifications and those still in the early stages of research, and discusses how they can be used to optimize biological interactions and improve clinical outcomes. Depending on the requirements of specific applications, implanted materials, including metals, ceramics and polymers, have found applications in a variety of medical disciplines. Titanium and its alloys as implant materials play a key role in orthopedic and dental procedures. However, they still require the use of surface modification technology to not only achieve solid osseointegration, but also enhance antimicrobial properties, which can help avoid implant-related infections [3,4]. Hence, surface modification is critical to the development and design of new biomaterials and medical devices. One of the most popular surface modification methods is physical vapour deposition (nanoPVD) and atomic layer deposition (ALD). ALD allows thin layers to build up layer by layer through alternating surface saturation reactions between the gaseous precursors and the substrate. The self-limiting nature of the ALD surface reaction provides thickness control at the angstrom level as well as exceptional layer consistency on complex structures [5].
Important in treatment of the skeletal system and the osteointegration process is that the material should be with a high level of biocompatibility and Young's modulus similar to the value of bone tissue. Therefore, the aim of this study was to assess the impact of the physicochemical and mechanical properties of the modified Ti13Nb13Zr alloy.

Materials and Methods

The initial state of Ti13Nb13Zr were polished by Al_2O_3 solution. In the next step the polished samples were deposited tin oxide by nanoPVD and ALD method in different variants. In order to assess to suitability of the surface modification method, the pitting corrosion resistance tests and electrochemical impedance spectroscopy (EIS) were carried out. These tests provided information concerning the structural characteristics of the layers, possible defects, lack of sealing, substrate reactivity and the presence of barrier properties involving the electrolyte. In the research observation by scanning electron microscope, surface wettability tests, scratch test and tribological tests were also completed, which showed the differences resulting from temperature changes and the number of cycles and their impact on individual parameters tested.

Results and Discussion

The obtained data showed different physicochemical properties of layers generated under different parameters. These results directly assist the optimisation of the tin oxide layer creation process using ALD-based methods and nanoPVD-method on surfaces of Ti13Nb13Zr alloy implants intended for skeletal system, thus improving their functional properties. The results have obtained can be used as a base to develop more detailed criteria of final quality of medical devices which will ensure the required biocompatibility of implants. It has contributed the risk mineralization of postoperative complications. As a result, it has increased effectiveness, decreased the indicator of complications and improved life of the patients.

Conclusions

Based on the obtained results, different physicochemical properties of the alloy with tin oxide layers depending on the number of cycles used and the temperature of the manufacturing process were found. The knowledge obtained on this basis is of practical importance for the application of this type of surface modification for various types of miniaturized implants that are used in the skeletal system.

Acknowledgments

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THE INFLUENCE OF THE MAGNETIC POWDER SILANIZATION TECHNIQUE ON THE SURFACE TOPOGRAPHY OF MAGNETIC SILICONE COMPOSITES

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Introduction

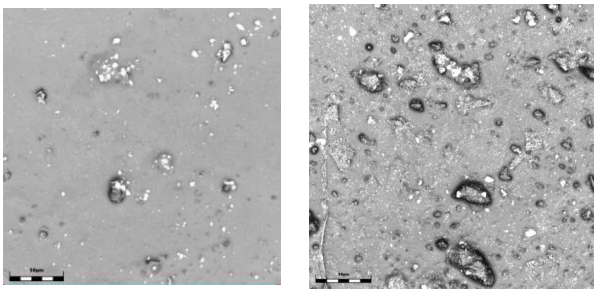
Elastic silicone-based composites with magnetic properties are a constantly developing group of smart materials. Composites reinforced with strong neodymium magnet micropowder are widely used in electronics and automatics [1]. There is a great potential of application of this group of materials in biomedical engineering. Our preliminary studies [2] shown that during their incubation in physiological conditions, elements were released to the contact solution. One of the directions to improve the micropowder-silicone adhesion is silanization. During this process, chemical functionalization of the powder surface is performed [3]. Moreover, silanization should prevent powder agglomeration [4].
The main goal of this work was an assessment of the influence of different silanization techniques on composite surface topography. The properties of surface are of crucial importance due to possible applications in biomedical engineering, where the topography plays significant role in biofilm formation.

Materials and Methods

In this study, silanization processes on the NdFeB micropowder (Magnequench, Singapore, Singapore) were carried out. The silane used in this study was 3-Aminopropyltriethoxysilane (APTES), and together with toluene and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Various techniques, including different silane percentages, solvents and silanization times were performed. Obtained powders were used as a reinforcement to silicone-based elastic composites. Materials consist of 70 wt.% of NdFeB powder and 30 wt.% of Ecoflex 30 silicone (Macungie, PA, USA). The preparation of the materials was performed using the method described earlier [5].
The composite surface topography, profile (Ra, Rz) and area roughness (Sq, Sa, Sp) parameters were measured and analysed using Olympus LEXT OLS 4000 confocal laser scanning microscope (CLSM) (Tokyo, Japan).

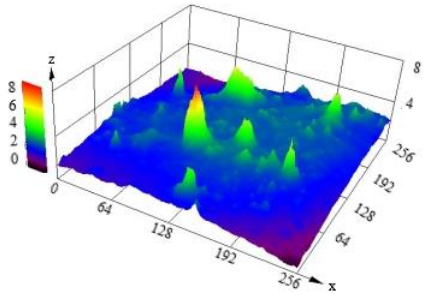
Results and Discussion

The results obtained for composites with silanized micropowder showed that silanization decreases the linear roughness Rz parameter. For composite with non-silanized micropowder the average Rz value is of 1.58 µm and for composites reinforced with silanized powder by different techniques Rz is in range 0.05-0.28 µm.
The surface roughness was also tested in this study. The Sq parameter was of 0.835 µm for composite with non-silanized micropowder and between 0.02 µm and 0.116 µm for composites reinforced with silanized powder by different techniques.



(a) (b)

FIG. 1. CLSM images of composite surfaces after (a) silanization with 5% silane for 4 h in ethanol/water 95/5 solution, (b) silanization with 5% silane for 4 h in toluene. Scale bar ~50 µm.



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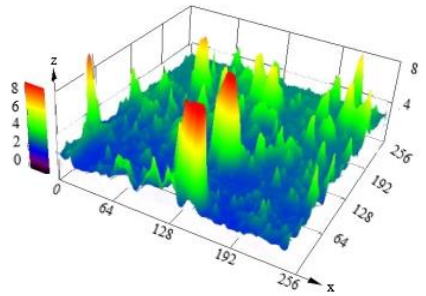

(b)

FIG. 2. CLSM 3-dimensional image of composite surfaces after (a) silanization with 5% silane for 4 h in ethanol/water 95/5 solution, (b) silanization with 5% silane for 4 h in toluene.

Conclusions

The silanization process, using various techniques, influences the topography of the composite surface. The value of roughness decreased in most of composites after silanization. This may have a positive effect on the surface energy, which is of high significance for regulation of biofilm growth kinetics.

Acknowledgments

This scientific work was realized in the frame of works, No. WZ/WM-IIB/2/2020 and WI/WM-IIB/5/2021 and financed from research funds of the Ministry of Education and Science, Poland.

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MODIFICATION OF THE HYDROXYETHYLCELLULOSE SHEAR RHEOLOGY AND WETTABILITY BY CATIONS

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Introduction

Hydroxyethylcellulose (HEC) is a gelling and thickening agent derived from cellulose, widely used in various industries. It is also a promising polymer in biomedicine for drug delivery systems like gels as well as micro- and nanocapsules [1]. The form, consistency and rate of drug release from these systems is determined by the rheological properties of HEC [2]. In this paper, we presented how the concentration of HEC and the addition of ions (Na^+, Mg^{2+}, Cu^{2+}, Al^{3+}) affect the rheological properties of HEC-based gels, which can be used in the design of drug carriers.

Materials and Methods

In this study, the rheological properties of prepared HEC solutions (1, 2, 3%$_{mas.}$) with different cations concentrations were measured on a parallel-plate, strain-controlled, rotational shear rheometer (HAAKE Rheostress 6000, Thermo Fisher Scientific, USA) (FIG. 1). The volume of each sample for rheological studies was 1 mL and the upper platen diameter was 35 mm. The rheological testing protocol consisted of: 1) oscillating shear strain with a frequency f = 1 Hz and an amplitude γ = 1% for 60 s at each compression level (ε = 0-20% with 5% steps); 2) amplitude sweep in the range of 0.01-1 at a frequency of 1 Hz; 3) sweep the temperature in the range of 30-60 Celsius degrees.
The wettability of the materials surface was determined using Contact Angle Goniometer (Ossila, Sheffield, UK). Wettability is defined by the contact angle (Θ) between the surface of the examined material and a droplet of ultrapure water on the surface.

Results and Discussion

Our studies have shown that the concentration and addition of cations in HEC-based gels significantly affect the rheological properties and wettability. FIG. 2a shows the results of amplitude sweep of gels with different HEC concentrations. HEC based gels show shear-softening. The higher the HEC concentration, the higher the storage G' and loss G' modulus values - it may affects the rate of active substances release. The ratio of these modules also changes - at lower concentrations, the gels show a more viscous character, while at higher concentrations, they are more elastic. This phenomenon is important while maintaining appropriate lubrication on the surfaces of tissues and implants *in situ* application. FIG. 2b shows the effect of ions on the value of G' storage modulus. Significant differences were observed for Cu^{2+} and Al^{3+}, which increase the stiffness of the gel.

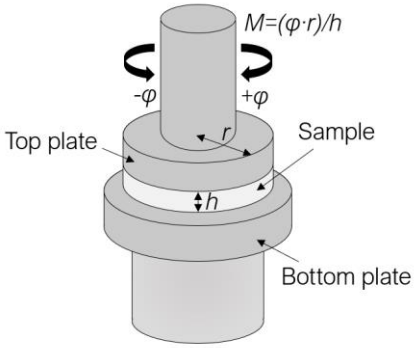
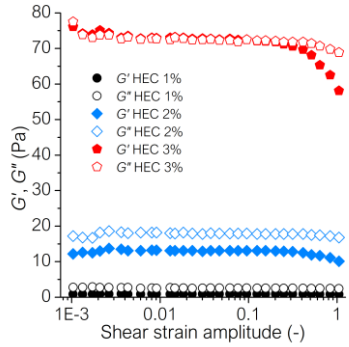
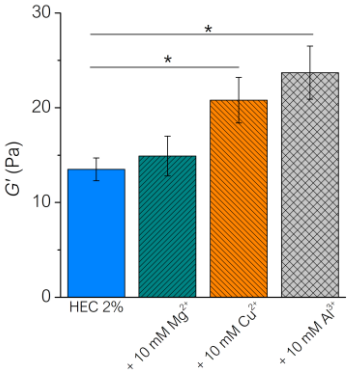


FIG. 1. Scheme of the rheometer measuring system. The shear of the sample is caused by the oscillating movements of the upper plate. Compression in the sample was achieved by changing the height between the plates.



(a)



(b)

FIG. 2. The rheological properties of HEC-based gels (a) G' and G'' during amplitude sweeping, (b) the effect of the addition of cations on G', *statistical significance ($p<0,05$).

Conclusions

The rheological properties and wettability of HEC-based gels can be modified by the concentration of HEC in the solution and the addition of cations.

Acknowledgments

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MAGNETIC POLY(ε-CAPROLACTONE)-BASED AND TANIC ACID-MODIFIED NANOCOMPOSITES FOR BONE REGENERATION

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Introduction

Modification of polymer scaffolds is a way to increase their functionality and boost the bone tissue regeneration [1]. In this study, poly(ε-caprolactone) (PCL) was modified with magnetic nanoparticles and biologically active tannic acid (TA). Magnetic field will stimulate cell proliferation and differentiation, whereas TA will enable collagen crosslinking, upregulate bone formation markers, inhibit activity of osteoclasts to prevent osteoporosis, and have an anti-inflammatory effects [2].

Materials and Methods

Magnetic iron oxide-based nanoparticles with grafted PCL (MNP@PCL) were synthesized by coprecipitation and ring-opening polymerization of ε-caprolactone from the surface. PCL-based nanocomposites containing MNP@PCL and/or TA were prepared by an electrospinning method. Series of composites containing 2 wt.% of MNP@PCL and different concentrations of TA (1, 2, 5, and 10 wt.%) were obtained. The nanoparticles were characterized using TEM, ATR-FTIR, and TGA. The effect of MNPs and amount of TA on physicochemical properties of composites, relative to PCL, was investigated by FTIR, SEM, tensile strength tests, etc. Antioxidant properties were evaluated by a Folin-Ciocalateu assay, while cytotoxicity of composites was determined on SAOS-2 human osteosarcoma cells and monitored on the 1st, 3rd, and 7th day of cultivation.

Results and Discussion

Developed particles showed a spherical shape with D_n = 10 nm. ATR-FTIR spectra of MNP@PCL particles confirmed the presence of PCL bands at 2956 and 2884 cm^{-1} (asym. and sym. stretching of CH_2 groups), 1725 cm^{-1} (C=O stretching), 1241 and 1186 cm^{-1} (C–C and C–O stretching) [3]. According to TGA, the amount of PCL grafted on MNP surface was 87 wt.%. In the spectra of polymer composites, no bands typical for MNPs or TA were observed, which could be explained by their overlapping with the PCL bands. All nanocomposites were in the form of fibers with smooth morphology. Modification of PCL with either TA or MNPs alone increased diameter of fibers twice, compared to unmodified PCL. A strong correlation between the content of TA and fiber morphology was not observed. According to the Folin-Ciocalteu test, the antioxidant properties of composites correlated with increasing content of TA.

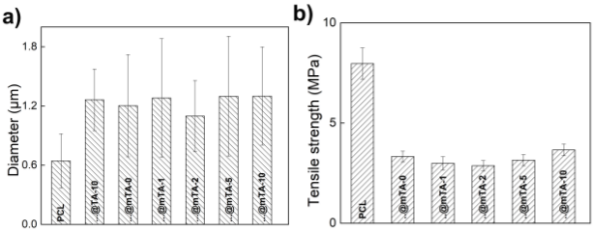

FIG. 1. (a) Size distribution of fibers and (b) tensile strength of unmodified PCL and nanocomposites.

Mechanical properties (Young's modulus and tensile strength) of composites correlated with size of fibers and were significantly deteriorated after the modification of PCL matrix with both MNP@PCL and TA alone. In the case of composites containing MNP@PCL and TA, a minor drop of mechanical properties was observed at 1 and 2 wt.% of TA, whereas a small enhancement was found at 5 and 10 wt.% of TA.

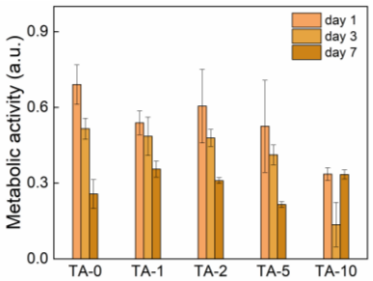

FIG. 2. Metabolic activity of SAOS-2 cells.

Microscopic observation revealed non-toxic character of nanocomposites, as well-spread cells were attached to the surface. Initially (day 1), the composites without TA induced the highest metabolic activity of SAOS-2 cells; however, on day 7, composites containing 1, 2, and 10 wt.% of TA led to higher metabolic activity than the composite without TA.

Conclusions

Modification of PCL with both MNP@PCL nanoparticles and TA significantly affected morphology and mechanical properties of composites. TA-modification improved the metabolic activity of SAOS-2 cells, compared to composite containing only MNPs. Developed materials can boost proliferation of bone cells, being thus promising for bone regeneration.

Acknowledgments

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SPIONs MODIFIED WITH β-CYCLODEXTRIN-PIOGLITAZONE COMPLEX FOR ANTI-CANCER THERAPY

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Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) possess various unique features, which make them a subject of the significant interest in scientific community. Among others, SPIONs found broad applications in medicine. Easy synthesis, high biocompatibility, possibility of surface modification and superparamagnetic properties, make SPIONs a valuable component of advanced drug delivery systems [1]. Recently they are also widely studied in the context of the circulating tumour cells (CTCs) capture and neutralisation [1,2]. Pioglitazone (PIO) is a ligand of peroxisome proliferator activated receptor PPAR-γ and was shown to possess the antitumor effect in several types of cancer. By activation of PPAR-γ, pioglitazone may inhibit cancer cell growth as well as so-called epithelial-mesenchymal transition (EMT), which is a process during which cancer cells gain the invasive character and metastatic potential [3]. The aim of our studies was to prepare colloidally stable SPIONs with attached anti-N-cadherin antibody and/or β-cyclodextrin-pioglitazone complex, which would be able to kill or capture colorectal cancer cells, especially those with metastatic potential.

Materials and Methods

Chitosan (low molecular weight, Sigma-Aldrich), glycidyltrimethylammonium chloride (GTMAC, ≥ 90%, Sigma-Aldrich), iron (II) chloride tetrahydrate (puriss. p.a., ≥98.5%, Sigma-Aldrich), iron (III) chloride hexahydrate (puriss. p.a., ≥98.5%, Sigma-Aldrich), p-toluenesulfonyl chloride (tosyl chloride, TsCl, ReagentPlus®, ≥99%, Sigma-Aldrich), β-Cyclodextrin (≥97%, Sigma-Aldrich) Human/Mouse/Rat 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, powder, BioReagent, ≥97.5% (HPLC), fetal bovine serum (FBS, qualified, heat inactivated, Gibco, Thermo Fisher Scientific), RPMI 1640 culture medium (Gibco, ThermoFisher Scientific) Human colon cell lines (American Type Culture Collection—ATCC, Manassas, Virginia, USA) - HT29 and SW620.

The average size and zeta potential of nanoparticles were measured using Zetasizer Nano ZS (Malvern Instrument Ltd.). Fourier-Transform Infra-Red (FTIR) spectra were recorded using Nicolet iS10 FTIR spectrometer with an Attenuated Total Reflectance (ATR) attachment (Thermo Fisher Scientific). ^{1}H NMR spectra were measured using Bruker BioSpin GmbH. Magnetic properties were determined using Vibrating Sample Magnetometer and 57Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer with 50 mCi 57Co/Rh source.

HT29 and SW620 cell lines were cultured in supplemented RPMI 1640 and DMEM medium, respectively, with 10% (v/v) of FBS in the incubator (37°C, 90% humidity with 5% CO2). MTT assay was performed according to the manufacturers protocol.

Results and Discussion

Chitosan was modified using GTMAC according to the procedure described before [4]. CCh/SPION nanoparticles were obtained by co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of CCh and further purified using magnetic filtration. The resulting CCh/SPION had an average hydrodynamic diameter of 120 nm, according to the DLS measurements, and were colloidally stable (average zeta potential about 47 mV). β-cyclodextrin was modified with p-toluenesulfonyl chloride [7] and characterized using ATR-FTIR and ^{1}H NMR. The inclusion complexes of modified β-cyclodextrin with PIO were synthesized based on literature reports [5] [6]. Tosyl-β-cyclodextrin was then attached to CCh/SPION and PIO was complexed under previously established conditions. The obtained systems were characterized using DLS and ATR-FTIR, and their magnetic properties were also established. Preliminary cytotoxicity studies were performed using MTT assay.

Conclusions

SPIONs stabilized with cationic derivative od chitosan (SPION/CCh) [4], were functionalized with the inclusion complexes of modified β-cyclodextrin with PIO, which were previously prepared and characterized. Modified β-cyclodextrin was then bounded to the surface of SPIONs/CCh, and the interaction between SPION-bound β-cyclodextrin and PIO were studied. The obtained nanoparticles were characterized physiochemically and preliminary biological studies were performed on colon cancer cell lines derived from primary tumours (HT29 cancer cell line) as well as metastatic site (SW620 cancer cell line).

Acknowledgments

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ELECTROPHORETIC DEPOSITION AND CHARACTERISATION OF SnO_2/BIOACTIVE GLASS/ CHITOSAN MULTICOMPONENT COATINGS ON TITANIUM SUBSTRATES

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Introduction

Titanium and titanium alloys are important materials for bone implants due to their beneficial physico-chemical properties [1]. Unfortunately, they are bioinert and prone to the formation of biofilm on their surface. Hence, it is necessary to apply bioactive coatings with antibacterial performance, to improve their biocompatibility. Chitosan is one of the most popular natural polymers for the matrix of the bioactive coating due to its non-toxicity and high biocompatibility. The addition of bioactive glass into chitosan coatings may stimulate their osteoinductivity and osteoconductivity [2]. On the other hand, the addition of metallic compounds may induce antibacterial properties. Sn and its compounds, such as SnO_2, can constitute an interesting alternative to Ag, Zn or Cu to provide antibacterial properties [3]. The presented work aimed to develop multicomponent SnO_2/bioactive glass/chitosan coatings on Ti substrates by electrophoretic deposition (EPD), as well as characterisation of microstructure, surface topography and selected properties of coated titanium.

Materials and Methods

Commercially pure titanium (CP-Ti, Grade 1) was used as substrate material. In order to prepare the suspension for EPD chitosan (2 g/l) was dissolved in water with the addition of acetic acid (1 vol%) and stirred for 3 days. Then the solution was supplemented with ethanol (50 vol). In the next step, 1 g/l sol-gel glass (with chemical composition $49CaO\text{-}40SiO_2\text{-}6P_2O_5\text{-}5SrO$, in mol%) and 0.2 g/l SnO_2 nanoparticles (NPs) were added. The suspension was dispersed for 20 min and used for electrodeposition. The CP-Ti substrate was the cathode and the anode was an AISI316L steel plate during the EPD. Voltages from 5-20 V and times of 4-8 min were used during the process. The electrophoretic mobility of particles was measured by Laser Doppler electrophoresis. The BET method was used to determine the specific surface area of the glass. The microstructure of particles used for EPD and coatings was investigated by scanning- and transmission electron microscopy (SEM, TEM). The adhesion strength of coatings was investigated using the cross-cut tape-test according to ASTM D3359-17. The roughness of materials was assessed by an optical profilometer. The contact angle (CA) and surface free energy (SFE) were measured with a goniometer, using water as a polar and diiodomethane as a non-polar liquid.

Results and Discussion

Sol-gel glass with a particle size up to 4.7 µm had an average pore size of 15 nm, pore volume of 0.16 cm^3/g and BET surface area of 37.6±0.1 m^2/g. Incubation of glass in the SBF solution revealed the formation of hydroxyapatite on its surface after 3 days of exposure. SnO_2 NPs with tetragonal primitive (tp) crystallographic structure exhibited a size up to 80 nm. Based on the zeta potential measurements of various suspensions the deposition mechanism was indicated. It relied on the adhesion of chitosan molecules on the surface of both bioactive glass and SnO_2 particles and their co-deposition on the cathode. It was found that macroscopically homogeneous coatings, without any cracks and voids, were obtained using a voltage of 10 V and a deposition time of 6 min. The coating has shown a well-developed surface and a high class of adhesion 4B, as shown in FIG. 1.

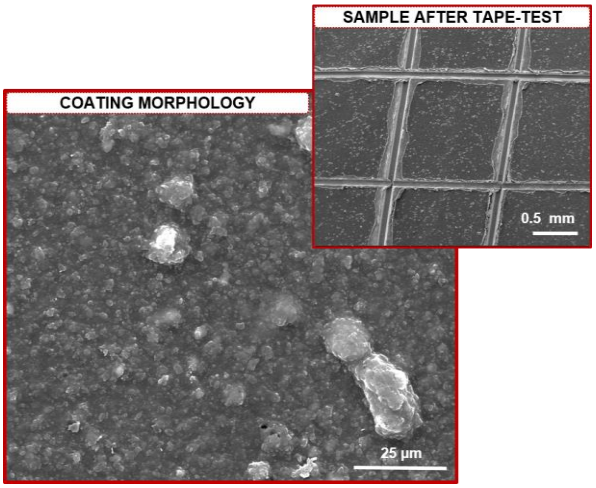

FIG. 1. The morphology and results of the adhesion test for the multicomponent coating on the CP-Ti substrate.

The SEM and TEM studies confirmed that the coating was rough and uneven in thickness, which was in the range of 1.5-2.5 µm. The roughness parameters showed higher values (Ra=0.47±0.04 µm, Rq=0.99±0.38 µm, Rt= 42±23 µm) in comparison with the CP-Ti substrate (Ra= 0.36±0.15 µm, Rq=0.46±0.2 µm, Rt=20±7 µm). The microstructure of the coating consisted of bioactive glass particles and SnO_2 NPs as well as agglomerates of both. The coating showed moderate hydrophilicity. The wettability tests revealed that lower contact angle (CA) values with water were obtained for the coating (49°±4°) than for the substrate material (77°±1°). The coating exhibited a higher SFE value of 64±2 mN/m than the substrate (44±2 mN/m).

Conclusions

In the presented work the multicomponent SnO_2/bioactive glass/chitosan coating on the titanium substrate was obtained and characterized. The chemical composition of the EPD suspension and the deposition process parameters, such as voltage and deposition time, were selected to obtain a macroscopically homogenous coating. The coating revealed high adhesion to the substrate and a well-developed surface. The hydrophilic character of the coating was detected. Further characterisation, including electrochemical corrosion resistance of the material, is in progress.

Acknowledgements

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FABRICATION AND CHARACTERIZATION OF HA AND HA/SOL-GEL GLASS COATINGS ON THE Co-28Cr-5Mo ALLOY

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Introduction

Co-Cr-Mo cobalt alloys are widely used in biomedical engineering, particularly for components subject to wear and friction. They are characterized by relatively high electrochemical corrosion resistance and outstanding mechanical properties. Due to high tribological properties, better than titanium alloys or austenitic stainless steel, currently, the latest application of cobalt alloys involves hip resurfacing systems [1]. Unfortunately, they show a very low level of osseointegration [2]. In order to improve it, in applications requiring strong and permanent adhesion of the implant to the bone cells, cobalt alloys are coated with bioactive materials such as calcium phosphates or bioactive glasses [3]. Hydroxyapatite (HA) is the major inorganic part of human bone, it exhibits both bioactivity and osteoconductivity, making it very attractive for biomedical applications. Bioactive sol-gel glasses are characterized by high porosity and a high surface area. Thus, it can be used as a material facilitating the reconstruction of bone tissue. Porous coatings allow for creating a very strong connection with the bone. This is called a biological connection that results from the ingrowth of bone tissues into the pores of the implant which does not require any additional fixation. A prospective method for the fabrication of porous ceramic coatings on metallic biomaterials is electrophoretic deposition (EPD). The aim of this work was the EPD of HA and HA/sol-gel glass coatings on the Co-28Cr-5Mo alloy substrates and the characterisation their microstructure and selected properties.

Materials and Methods

A Co-28Cr-5Mo alloy was used as the substrate material. The samples were in the shape of discs. Two suspensions for the EPD process were prepared, the first one contained 10 g/l HA powder, the second one contained 10 g/l 54CaO-6P_2O_5-40SiO_2 (in mol%) sol-gel glass powder. In both cases, ethanol with 99.8% purity was used as a dispersion medium. Directly before EPD, suspensions were ultrasonically dispersed for 10 min and magnetically stirred for 20 min. The EPD was carried out in a standard two-electrode system. The working electrode was a cathode and austenitic stainless steel was used as an anode. The distance between electrodes was 10 mm. The suspensions were magnetically stirred during the deposition at a speed of 20 rpm. The coatings were deposited at a constant voltage in the range of 10-30 V with a change of 5 V and a constant time of 60 s. During EPD, the current density with the deposition time was measured. To determine the influence of deposition time on deposit weight, coatings were deposited at a constant voltage of 20 V and a time in the range of 20-100 s with a change every 20 s. The samples were weighed after each deposition. Immediately after deposition and drying of the coatings, coated samples were subjected to heat treatment at a temperature of 850°C for 20 min. A heating rate of 10°C/min was used, and cooling was carried out with the furnace. Micro(structural) investigations of the materials were performed using scanning and transmission electron microscopy (SEM, TEM) and Fourier transform infrared spectroscopy (FTIR). The chemical composition was determined using energy dispersive X-ray spectroscopy (EDS) microanalysis. The scratch resistance of the coatings was examined in the micro-scratch test. The specific surface area of the glass was investigated by BET analysis. The changes in the concentration of Ca, P, and Si in a simulated body fluid (SBF) during incubation of the glass were evaluated by inductively coupled plasma atomic emission spectrometry (ICP-OES).

Results and Discussion

The microstructure of the Co-28Cr-5Mo alloy consisted mainly of the γ grains with a face-centred cubic (fcc) structure. The grains were recrystallized with the presence of numerous stacking faults and annealing twins, and their diameter was in the range of 4-15 μm. Microscopic investigation of HA powder revealed the presence of two types of spherical particles: nanoparticles (NPs) with a diameter up to 100 nm and microparticles with a diameter in the range of 0.2-0.6 μm. The sol-gel glass particles were characterized by a diameter up to 2.5 μm and showed a high surface area of 53 m^2/g with an average pore size of 10 nm. Structural analysis (FTIR) and changes in ion concentration (ICP) during immersion in SBF confirmed the bioactive properties of glass particles, evaluated as the ability to form a surface calcium phosphate layer. Macroscopic observations showed that both coatings deposited at a constant voltage of 20 V and time of 60 s were uniform. The microstructure of both types of as-deposited coatings was homogeneous, free from any cracks and voids. The microcrystalline HA and/or sol-gel glass particles were uniformly distributed within HA NPs and formed a matrix of the coatings. The HA coating after heat treatment showed open porosity. The particles were sintered and solid bridges were formed between them. Pores with a diameter in the range from a few nm to 1 μm were uniformly distributed in the coating. The HA/sol-gel glass coating after the heat treatment also showed a significant degree of porosity. However, numerous microcracks were also present. The coatings showed high scratch resistance, despite relatively high porosity. The removal of the coatings from the scratch track took place at loads of 22 N and 17.5 N for the HA and HA/sol-gel glass coating, respectively.

Conclusions

This study showed that electrophoretic deposition is effective for the fabrication of porous ceramic HA and HA/sol-gel glass coatings. In the coatings microstructure, micro and nano HA particles, as well as micro sol-gel glass particles, were present. As a result of heat treatment, the particles were sintered and formed solid bridges. Both types of coatings showed high scratch resistance, of which HA coatings were slightly higher.

Acknowledgements

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EFFECTS OF BLOWING AGENTS AND ORGANIC SOLVENTS APPLIED IN SOLVENT CASTING PARTICULATE LEACHING TECHNIQUE ON SCAFFOLDS MICROSTRUCTURES

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Introduction

Tissue scaffold, by definition, is a three-dimensional network that mimics the extracellular matrix of bone tissue, the task of which is to provide optimal conditions for viability, attachment, growth, migration and osteogenic differentiation of cells, leading to full regeneration of damaged tissue in vivo [1,2]. The effectiveness of full mineralization of the regenerated tissue depends on a large extent on the morphology of the tissue scaffold, its resulting porosity, size, geometry and interconnection of pores, determined by the composition and the method of manufacturing the implant. Among all the challenges to tissue scaffolds, the role of porosity in bone engineering applications turns out to be crucial for the proper course of the tissue reconstruction process, strongly affecting not only the processes of attachment, multiplication and differentiation of bone tissue cells, but also influencing the permeability of the tissue scaffold for nutrients, its neovascularisation [3], an exponential decrease in the mechanical properties of the scaffold and the rate of its degradation [4]. For these reason, the aim of this study was a comparative assessment of the influence of blowing agents and organic solvents applied in Solvent Casting Particulate Leaching technique on scaffolds microstructure and thermal properties.

Materials and Methods

Scaffold fabrication. Scaffolds were produced using Solvent Casting - Particulate Leaching technique. In brief, 5g of PLLA was dissolved in 1,4-dioxane, dichloromethane or tetrahydrofurane at a concentration of 25% (w/w) by stirring with a magnetic stirrer until a homogeneous mixture was obtained. To the polymer/solvent solution an appropriate fraction of blowing agent (100-300 µm) in the form of sodium chloride, sucrose, potassium bicarbonate, sodium citrate or ammonium bicarbonate with concentrations ranging from 85 to 90% (w/w) were added. The prepared mixtures were transferred into molds and allowed to evaporate the solvent. In order to obtain a porous scaffold structure, the samples were subjected to porogen leaching process by immersion in deionized water at 40°C for 48 h.

Microstructure analysis. The microstructure of the produced tissue scaffolds was determined using JSM-6610LV Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) with the accelerating voltage of 20 kV.

Porosity determination. The porosity of the scaffolds was investigated using mercury intrusion porosimetry (AutoPore IV 9500 V1.07) in the pressure range of 0.58-45000 psia. Scaffold contact angle of mercury equaled 130°. Hg Surface Tension measured 485.000 dynes/cm, and Hg Density equaled 13.5335 g/mL.

Thermal properties analysis. Thermal properties were determined using Differential Scanning Calorimetry (NETZCH F1 204) with nitrogen as a sweeping gas. The samples were loaded in aluminum pans and subjected to heating processes in the temperature range from ambient to 200°C with heating rate of 10°C/min. Based on the obtained thermograms glass transition temperature (Tg), melting temperature (Tm) and crystallization temperature (Tc) were determined.

Blowing agent residual content determination. Thermogravimetric analysis was performed in order to determine the onset temperature of thermal degradation process and the residual concentration of sodium chloride particles in the PLLA-based scaffolds. Thermogravimetric evaluation was carried out using TGA 5500 (TA Instruments) at a heating rate of 10,00°C/min in the temperature range of 22-600°C. Residual weight at 600°C derived only from sodium chloride particles remaining in the polymer matrix.

Results and Discussion

Produced scaffold were characterized by a porous structure characteristic for the Solvent Casting Particulate Leaching technique with the observed average pore size in the range of 2-200 µm. The produced samples exhibited porosity above the 60%. Therefore, these values are within the range 60-90% reported in the literature data as the most desirable for bone scaffolds applications [3]. Thermal analysis shows that the determined melting temperatures of pure PLLA and PLLA- based scaffold prepared by SCPL technique were similar. The TGA curves obtained for all samples showed an onset temperature of thermal degradation process in the range of 216-256°C, indicating that the application of different solvents during the scaffolds fabrication process did not significantly affect the thermal stability of the samples. The residual porogen content for all samples was less than 3%, what indicates the high efficiency of the particle leaching process procedure.

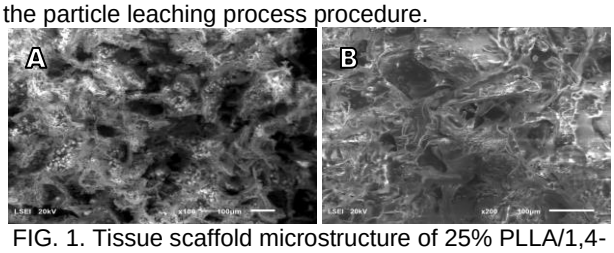

FIG. 1. Tissue scaffold microstructure of 25% PLLA/1,4-dioxane + 88% potassium bicarbonate (A) and 25% PLLA/dichloromethane + 86% sodium citrate (B).

Conclusions

The applied parameters allowed to obtain the porous microstructure with the porosity degree in the range required in the field of bone tissue engineering. The use of different types of organic solvents in the production processes did not affect the significant differences in the analyzed thermal properties of the samples and the residual sodium chloride content was acceptable. However, it is necessary to develop a procedure to determine the remaining amount of organic blowing compounds in the structure of the produced samples.

Acknowledgments

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BIOMATERIALS FUNCTIONALISED BY COVALENTLY BOUND PROTEASE INHIBITORS – ANTIMICROBIAL AND CYTOTOXICITY ANALYSIS

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Introduction

Microbial infections of the vascular prosthesis are still a serious threat to health and life of many people. Therefore, in the field of biomaterial engineering, new solutions that will allow increasing the aseptic potential of implanted prostheses are still being sought [1]. Inhibitors of serine proteolytic enzymes have been proposed as an innovative solution for the limitation of the adhesion of pathogenic microorganisms to the surface of prostheses through inhibition of biofilm formation and inhibition of microbial multiplication [2].

Materials and Methods

The main goal of the study was to analyse the biological properties of polymeric biomaterials modified with two serine protease inhibitors – α1-AT - human α1-antitrypsin (natural peptide) and AEBSF - 4-(2-aminoethyl) benzenesulfonylfluoride (low molecular-weight synthetic compound). Three commercially available prostheses were used: (1) the Uni-Graft® K DV prosthesis – a knitted gelatine-impregnated polyester vascular prosthesis, (2) the Hemagard Intergard prosthesis – a knitted polyester vascular graft impregnated with collagen, and (3) the skull bone Codubix prosthesis – a polyester-polypropylene prosthesis with mechanical properties similar to natural bone. The inhibitor molecules were covalently immobilized [3] on the surface of polymeric biomaterials using glutaraldehyde (GLA) as a cross-linker. The antimicrobial activity of modified biomaterials was tested on the following microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, with particular emphasis on *S. aureus* interaction with the modified prosthesis preparations in the first 24 hours of contact. The cytotoxicity against the cell lines HSF - human skin fibroblasts and HUVEC - human umbilical vein endothelial cells, was also analysed.

Results and Discussion

Among the proposed preparations of modified biomaterials, the most intense inhibition of microbial growth related to the presence of proteolytic enzyme inhibitors was observed in the case of three of the four tested strains: *S. aureus, P. aeruginosa,* and *C. albicans*, which were clinical isolates. The immobilization process increases the inhibitors' activity and their stability in different pH and temperature values. The rate of proteolytic enzyme inhibitors released from the surface of modified biomaterials was low.

The highest anti-biofilm activity was observed against *S. aureus* strain which is responsible for the vast majority of vascular prosthesis infections [1]. For example, the Uni-Graft prosthesis surface modification using the synthetic AEBSF inhibitor had a significant effect on the limitation of the *S. aureus* proliferation and contributed to the inhibition of the production of normal mature biofilm structure already in the first hours of the microorganism contact with the biomaterial's surface. The reduction in the number of microorganisms on the modified prosthesis and their metabolic activity (TTC test) during the first 6-12 hours of incubation were observed. Importantly, the control prosthesis (polymeric biomaterials activated with GLA, but without the attached inhibitor molecules) showed no cytotoxicity against the tested cell lines, nor increased bactericidal/bacteriostatic properties against the tested microorganism strains. The use of protease inhibitors in this work as factors limiting the formation of biofilm is most likely based on the inhibition of microbial cell proliferation and the reduction of cell aggregates formation surrounded by the extracellular polysaccharide matrix [4].

The introduction of the modifications on the surface of the Uni-Graft prosthesis using both the AEBSF and α1-AT inhibitors did not reduce the adhesion potential of HUVEC epithelial cells and HSF cells. The surface of the unmodified and modified prostheses was densely populated with epithelium cells, and after 5 days from the beginning of the culture, the number of cells increased. The cytotoxic effect of the modified Hemagard and Codubix prostheses on HSF cells was observed due to their weaker adhesion properties in the case of epithelial cells. This may be caused by the presence of large pores in the material structure. Extensive research has confirmed that the porosity of the material promotes cell adhesion; however, Hemagard and Codubix prostheses probably have too large pores for the investigated cell lines (related to the method of weaving and manufacturing the prostheses), which makes the proper adhesion process difficult.

Conclusions

The modification of biomaterials with proteolytic enzyme inhibitors is a promising area of research and application. The analyses of the biological activity against *E. coli, P. aeruginosa, S. aureus*, and *C. albicans* strains have shown that biomedical materials modified with the model proteolytic enzyme inhibitors can inhibit the proliferation of microbial cells and limit biofilm formation on the biomaterial surface. The highest activity in contact with liquid cultures was observed in the case of the Uni-Graft prosthesis modified with the synthetic inhibitor AEBSF and α1-antitrypsin against the *P. aeruginosa* and *C. albicans* strains and so functionalised Uni-Graft prosthesis showed no cytotoxicity towards the HSF and HUVEC cell lines, which is very promising in the context of further research on the potential use of proteolytic enzyme inhibitors in vascular medicine. Additional interesting aspect that should be investigated in the future is the effect of protease inhibitor-modified vascular prostheses on the blood coagulation process. This issue is crucial, as new biomaterials designed for patient safety should demonstrate anti-thrombogenic properties.

Acknowledgments

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OPTIMIZATION OF THE PREPARATION PROCESS OF A SODIUM ALGINATE AND GELATIN-BASED BIOINK TO IMPROVE THE VIABILITY OF CELLS CONTAINED IN THE PRINTOUTS

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Introduction

The possibilities of large-scale 3D bioprinting are creating promising solutions for tissue engineering and personalized medicine [1]. Hydrogels based on sodium alginate are characterized by high biocompatibility, water and gas permeability and mild crosslinking conditions. The possibility of modifying the rheological properties makes alginate-based hydrogels ideal substrates for bioinks used in 3D bioprinting. However, alginate hydrogels do not contain groups that stimulate cell adhesion and proliferation, which results in their biological inertness [2-4]. To overcome the limitations of alginate, it is often used in combination with gelatin, which contains in the structure bioactive RGD (arginine-glycine-aspartic acid) sequences that promote cell adhesion and proliferation [2,4-6]. The high viability of cells contained in the bioink and in the printed structures is a key factor in determining the applicability of the hydrogel material. The aim of this study is to develop a protocol for the preparation of alginate- and gelatin-based hydrogel bioink to ensure the highest viability of the cells contained in the bioink before the 3D bioprinting process. A key aspect of the research is to evaluate the impact of the 3D printing process on the cell viability immediately after the manufacturing process and after printouts incubation.

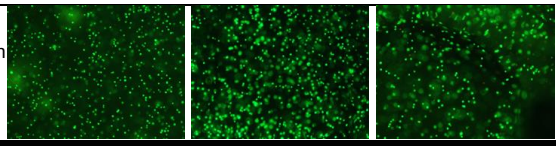
Materials and Methods

Sodium alginate (Alg) and porcine skin type B gelatin (Gel) powders were sterilized by UV light for 1 hour, then dissolved separately in culture medium McCoy's 5A supplemented with 15% FBS and 1% P/S antibiotics for 1 hour at 37°C at 150 rpm to obtain solutions of 4% w/v Alg and 18% w/v Gel. Polymer solutions were combined in a 1:1 ratio to obtain a composition of 2% w/v Alg, 9% w/v Gel. The two-component hydrogel was combined with immortalized model cells line *Saos-2* and poured to form equal height samples, which were crosslinked using calcium chloride solution for 10 min. The effect of the preparation process of hydrogel bioinks on the viability of cells was evaluated by analysing parameters such as: concentration (0.75% 1.5%, 5%) and solvent (McCoy's 5A culture medium or deionized water) of calcium chloride crosslinking solution, method of combining cell pellet with polymer solution (direct combination of hydrogel with cell pellet, initial combination of cell pellet with 2% or 8% of the volume of the culture medium used to prepare the hydrogel, and then mixing the cell suspension with the hydrogel by pipetting) and the waiting time (0, 30, 45 minutes) of the hydrogel bioink for the 3D printing process. The final part of the study was the evaluation of 3D printing process effect on the changes in cell viability during subsequent incubation periods of the printouts under standard conditions. The 3D bioprinting process was performed using a custom designed bioprinter. The hydrogel was extruded to form tubular structures with 6 mm the diameter using a 250 µm conical nozzle at 34ºC. To assess the viability of cells placed in the samples it was used the Live/Dead assay. Statistical significance was evaluated using one-way ANOVA analysis of variance. Statistical significance was indicated in the graphs when $p < 0.05$ by an asterisk.

Results and Discussion

The analysis of the effect of the solvent and the concentration of the hydrogel crosslinking agent indicated that the use of $CaCl_2$ crosslinking solution prepared in McCoy's 5A culture medium leads to an increase in the cell viability in relation to solutions of $CaCl_2$ in deionized water. This phenomenon may be a result of the interaction of calcium ions with the components contained in the culture medium. The study demonstrated that the use of 5% crosslinking solution prepared in the culture medium allows to obtain analogous cell viability (88.0±2.1%) in comparison to the hydrogel crosslinked with 0.75% $CaCl_2$ solution (88.2±2.0%) while allowing to obtain a material with a higher crosslinking degree and higher stability. The results of the analysis of the effect of the method of combining the hydrogel material with the cell pellet demonstrated that the highest cell viability (90.9±1.6 %) was characteristic for the samples in which the cells were suspended in the highest volume of the culture medium used for hydrogel preparation. Analysis of the effect of the bioink waiting time on the 3D bioprinting process on cell viability indicated that extended to 30- or 45-minutes waiting time did not reduce cell viability in the final printouts. Cell viability after the 3D printing process for all analyzed bioinks was above 97%. During the increasing incubation time of the printouts, the cell viability decreases. After any of the degradation periods it does not take the value below 70%, which according to ISO 10993 standard indicates nontoxicity of the material. The decrease in the viability of cells contained in the printouts may be a result of the release of calcium ions crosslinking the hydrogel and gelatin, which becomes liquefied at 37°C.

Table 1. Example results of evaluation of cell viability in hydrogel printouts after individual periods of incubation using the Live/Dead test.

Incubation time [days]	0	1	3
Evaluation of cell viability			

Conclusions

The selection of parameters for the preparation and crosslinking process of alginate- and gelatin-based hydrogel bioinks has a key impact on the viability of the biological material contained in the hydrogel printouts. The 3D bioprinting process does not reduce the cell viability for the developed bioink composition. With increasing incubation time of the printouts, the cell viability gradually decreases. Further experiments are needed to analyze and evaluate of the changes in cells viability and proliferation for longer printouts incubation periods.

Acknowledgments

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WATER SORPTION AND SOLUBILITY OF DENTAL COMPOSITES MODIFIED WITH LIQUID RUBBER

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Introduction

The amount of water absorbed by the composite depends mainly on the reinforcement content and the hydrophilicity of the resin. Although the polymer matrix is insoluble in water, the diffusion of water therein may cause it to swell as a result of the affinity of water for certain functional groups or bonds contained in the network, e.g. hydroxyl groups and ester or ether bonds [1]. This in turn leads to a deterioration of the mechanical properties by plasticizing the matrix. The amount of absorbed water and its diffusion are conditioned, among others, by conversion rate value [2]. These effects are more pronounced when the conversion rate is low and lead to increased abrasive wear [3]. BisGMA resin, as the main component of the matrix of composites, has polar hydroxyl groups [4], while liquid rubber is non-polar [5], which may favor increasing the contact angle and water sorption. The aim of the study is to assess the water sorption and solubility of composites modified with liquid rubber.

Materials and Methods

Commercial light-cured dental composites Flow-Art and Boston marked F and B, respectively, were used as reference materials. The test material was analogous composites modified with Hypro 2000X168LC liquid rubber (Huntsman International LLC, USA). These samples were labeled FM and BM, respectively.

For the research of water absorption and solubility, 10 samples of each material were prepared. The research methodology complied with the ISO 4049 standard. Briefly, 1) samples were dryed at a temperature of (37±1)°C, to a constant mass m_1; 2) next step was incubation in distilled water at the temperature of (37±1)°C for 7 days, mass m_2 was measured; 3) drying the samples at a temperature of (37±1)°C, to a constant mass of m_3. The geometric measurements of the samples allowed to calculate their volume V. Water sorption W_{sp} and solubility W_{sl} was calculated according to formulas:

$$W_{sp} = \frac{m_2 - m_3}{V}; W_{sl} = \frac{m_1 - m_3}{V}$$

Statistical significance was considered at a probability of $p<0.05$ using the Statistica software (TIBCO Software Inc.).

Results and Discussion

The averaged results of measurements of the mass change (percentage) with water sorption are presented in FIG. 1. A dynamic increase in mass was observed in the first 2 days of incubation, then the growth rate decreased until reaching the saturation state. Both modified composites presented lower water sorption.

The results of water sorption determinations are presented in FIG. 2 and 3. Results indicate a decreasing tendency of water sorption after rubber modification, but the differences between the mean values for the pairs of materials F and FM as well as B and BM were not statistically significant. Likewise, the solubility results indicate a decreasing tendency after liquid rubber modification, however again without statistical significance.

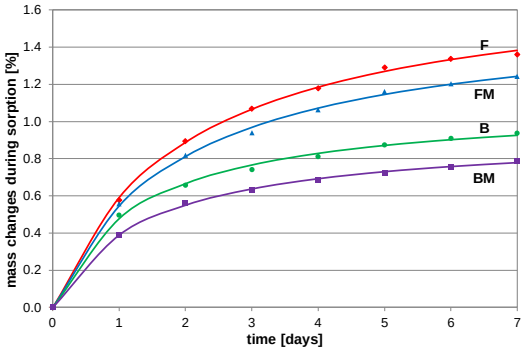

FIG. 1. Percentage mass change in water sorption.

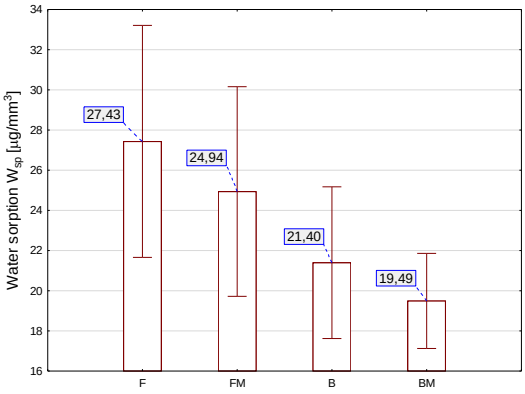

FIG. 2. Water sorption W_{sp}.

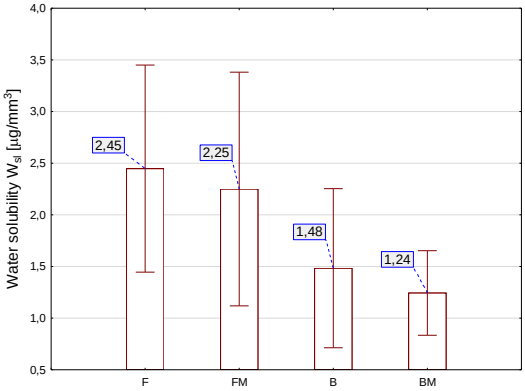

FIG. 3. Water solubility W_{sl}.

Conclusions

Modification of light-cured dental composites with liquid rubber does not significantly change water sorption or solubility, however, there is a downward trend for both properties after the modification was applied.

Acknowledgments

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BIODEGRADABLE SCAFFOLDS BASED ON IRON FOR POTENTIAL APPLICATION IN ORTHOPEDIC

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Introduction

Recently, research has been performed on implants that have the ability to biodegrade. They could be an alternative to the currently used implants, which remain in the human body permanently, even when they are no longer needed [1]. Achieving optimal regeneration requires that the implanted material as a scaffold complies with certain morphological and mechanical requirements. One of the most important morphological characteristics of implants is their porosity. The pores in the structure provide a channel for the transport of body fluids and stimulate proliferation and attachment of new tissue [2]. Metals such as stainless steel, titanium and titanium alloys are widely used in the field of surgery due to their excellent mechanical properties. However, the release of their ions can have a negative effect on the body in the long term. Additionally, their removal requires another surgical procedure, which puts a stress on the patient and increases the cost of treatment [3]. Degradable biomaterials appear to be able to overcome these disadvantages and may seek to replace those currently used in implantology. One of the elements that may have applications in these biodegradable systems is iron. Iron-based materials combine high strength with medium corrosion rates [4].

Materials and Methods

The macro-porous Fe scaffolds were made of iron powders particle sizes <10 micron. The powders were mixed with a solution of 5% polyvinyl alcohol in a mass ratio of 1:2. And then the templates made of polyurethane PU PPI45 were soaked and squeezed out. The samples prepared in this way were placed in a tube furnace and sintered for 5 h in II steps (I step from RT to 500°C with rate 8°C/min, II step to 1050°C with rate 6°C/min). The morphology of the produced scaffolds was studied using a Quanta scanning electron microscope with field emission. Phase homogeneity was confirmed using X-ray diffraction. Corrosion behaviour was determined using immersion and potentiodynamic polarization methods in phosphate buffered saline (PBS). The surface energy was calculated by studying the changes of enthalpy of calorimetric immersion.

Results and Discussion

The iron scaffolds produced are similar in architecture to a sponge, with a pore diameter of 245-360 μm. The macro pore size obtained is very close to the pore size suggested for bone regeneration [5]. The results of EDS analysis confirm the purity of the obtained scaffolds. Moreover, XRD analysis showed that the obtained signals on the diffractogram correspond to the standard of pure iron. An intense (110) diffraction peak was obtained along with characteristic signals at (200), (211), (220) and (310) [6]. Due to the presence of macropores in the prepared structure, it proved impossible to perform a classical wetting angle measurement, so we used the measurement of the enthalpy of immersion in different liquids to characterise the surface energy components using the van Ossa-Good-Chaudhury (VGC) approximation. The model used separates the acidic H_S^+ and basic H_S^- components, which not only allows the calculation of the total surface energy, but also provides information about the interactions of adsorbents with molecules on the surface. From the obtained calculations, the H_S^+ is 33 times larger than the H_S^-, which proves that the obtained iron scaffold is more of an acidic material than a basic one. Furthermore, electrostatic interactions dominate compared to non-polar interactions. The high water count and adhesion work prove the good wettability of the material in the natural tissue environment [7].

The immersion tests performed in PBS solution at 37°C showed that an increasingly rapid weight loss of the sample was observed with each week. The highest weight loss was observed after 4 weeks, at which time the samples started to disintegrate. The corrosion rate results obtained for porous iron samples are higher than for non-porous ones, which may be related to the increased surface area, macropores affecting the increased solvent access to the material and the roughness of the sample [8].

Conclusions

Pure iron scaffolds with macroporous pore size, obtained with the use of template method, appear to be a promising candidate for biodegradable bone implant material. They showed a suitable pore size, almost ideal for a bone substitute material. Nevertheless, further work is needed to assess the rate of biocorrosion, to check biocompatibility with cell lines and to verify possible cytotoxicity.

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NEW COMPOSITE MATERIALS BASED ON Ti(IV) COMPLEX WITH ASPIRINE LIGANDS

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Introduction

The interest in the synthesis of oxo-titanium(IV) multinuclear complexes results, among others, from their unique physicochemical and optical properties and potential application in various modern technologies [1]. Acetylsalicylic acid (aspirin—asp) is a commonly used agent, with antipyretic, anti-inflammatory, and anticoagulant effects. The biological activity of aspirin is due to the actions of the molecule's acetyl and salicylate portions. Therefore, it was interesting to investigate the possibility of using this compound to synthesize Ti(IV)-oxo complexes [2]. Due to the fact that systemic administration of drugs may cause side effects, studies have been undertaken to develop a method of local administration. Torsney et al. [3] showed that local application of aspH reduces thrombus formation in venous transplants. Subsequent studies [4] showed that the topically effective complex of acetylsalicylic acid and ticagrelor UFH reduces thrombus formation in venous transplants compared to other treatments. In our research, we propose the synthesis of a new group of anticoagulants (coordination complexes with ligands that already have anticoagulant properties). The presented results contain information on the structure of the new compound of titanium(IV) oxo-complex with acetylsalicylic acid (TOC) and photocatalytic studies of the produced composite materials (Polycaprolactone + TOC). A poly(ε-caprolactone) (PCL) is a biodegradable thermoplastic polymer increasingly used in the production of medical devices [5]. Since the PCL matrix may undergo degradation in water surroundings, it was important to study the stability of the composite sample during photocatalysis processes.

Materials and Methods

The synthesis of the tetranuclear Ti(IV)-oxo complex stabilized by acetylsalicylic ligands (1) was carried out as described in [6]: 0.16 g of acetylsalicylic acid (0.875 mmol) was added to the solution of 1.19 mL titanium(IV) isobutoxide (3.5 mmol), in 2 mL of THF/HOiBu (1:1), leading to a clear yellow solution. The solution was left for the crystallization. The light yellow crystals of $[Ti_4O_2(asp)_2(Bu^iO)_{10}]\cdot H_2O$ were isolated from the mother liquor after three days; yield: 74%.
The composite films containing 10, 15, and 20 wt.% of isolated Ti(IV)-oxo complex micrograins were prepared, by an addition of (1) (0.10, 0.15, and 0.20 g) dispersed in 1 mL of THF to the polycaprolactone (PCL) solution (1.0 g of PCL dissolved in 5 cm^3 of THF). The resulting mixtures were stirred in an ultrasonic bath for 120 min. In the next step, they were poured into a glass Petri dish, and left for the evaporation of the solvent at RT in the inert atmosphere (glove box).

Results and Discussion

During the investigations carried out, a $[Ti_4O_2(O^iBu)_{10}(asp)_2] \cdot H_2O$ (1) cluster was synthesized, the structure of which was solved by single-crystal X-ray diffraction. The oxo complex (1) was also characterized by vibrational spectroscopy (IR and Raman), and solid state 13C NMR spectroscopy. The results of the conducted research confirm that the use of a 4:1 molar ratio (titanium alkoxides and organic acid), and the above-mentioned reaction conditions, leads to the formation of oxo cluster, which consists of $\{Ti_4O_2\}$ cores.
Analysis of the UV–Vis DRS spectrum proves the maximum absorption position at c.a. 395 nm, and the value of the HOMO–LUMO energy band gap on the level 2.35 eV. This energy bandgap decrease is important for the photocatalytic activity of this compound, especially for its shifts towards the visible range. Therefore, in all our photocatalytic experiments, samples were irradiated by visible light between 395 and 425 nm. In studies on photocatalytic activity, the possible sensitivity of (1) to hydrolysis processes causes the composite samples produced by dispersion of the oxo complex in the poly(ε-caprolactone) (PCL) matrix (PCL + (1)), which are then used. The photocatalytic experiments reveal good PCL + (1) film photocatalytic activity in the visible range. This activity does not change during the degradation process of the PCL matrix, which may result from the structural stability of the oxo cluster (1). Registering the IR spectra of PCL + (1) composite before and after 140 h of the photodecolorization process of the MB aqueous solution, clear differences are noted. Analysis of IR spectra, indicate that the structure of (1) during the whole photocatalytic experiment does not change. On the other hand, in the IR spectrum of the PCL + (1) composite after photocatalytic experiments (140 h), the effects associated with the beginning of PCL degradations appear.

Conclusions

The conducted research proved that it is possible to synthesize titanium (IV)oxo-complexes with such anticoagulant, as aspirin, using the method proposed by our team. The introduction of (1) microcrystals to the polymer matrix causes a significant shift of the absorption maximum from the UV range up to the visible range. The studied composite films reveal good photocatalytic activity toward MB; however, their activity slightly changes with the content of TOCs in the polymer matrix.

Acknowledgments

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MICRODEVICES WITH NANOSTRUCTURED ZnO FOR ELECTRICAL CELL STIMULATION

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Introduction

The ability of several types of cells to respond to electrical stimuli has largely been proved. Electrical signals are responsible of cellular processes - proliferation, differentiation, migration- and physiological processes, like nervous impulses and muscle contraction. In bioelectronics, electronic devices are used to deliver an electrical signal and activate these pathways in living cells. To safely deliver the electrical signal without the need of cables or highly energetic electromagnetic pulses -that alter the cells and their environment-, the use of piezoelectric materials is being implemented [1,2]. Ultrasounds (US) in the biomedical range (MHz) can be used as a wireless way of communication with the devices inside of our body [2], in combination to piezoelectric materials that generate an electric dipole along their surfaces when mechanically stressed [1].
We have developed a cell-scale microdevice with piezoelectric nanostructures through microfabrication techniques [3] and hydrothermal synthesis. The cytocompatibility of the microdevices of different sizes was analysed on osteoblasts. In addition, we are working on the effect of the electric fields generated by the microdevices on cells by stimulating them using ultrasonic pulses [1,2].

Materials and Methods

First, the microparticle template for the microdevices was designed following a microfabrication process in the clean room; generating three different microparticle array with microparticles of a size of 3 x 3 μm^2, 6 x 10 μm^2 and 12 x 18 μm^2. The metallic layer of aluminium nitride (AlN) served as *seed layer* for the piezoelectric zinc oxide (ZnO) grown in the form of nanosheets (NSs) through hydrothermal chemical synthesis [4]. The microdevices were peeled-off, washed several times and suspended finally in ethanol, ready for its forthcoming use.
Secondly, the microdevices were cultured with Saos-2 human osteosarcoma cells. The experiments performed encompass a cytocompatibility test at days 1, 3 and 7, using calcein and ethium iodide; also, an internalization assay, where the cells were stained with phalloidine (actine fibers) and Hoechst dye (nuclei). Cells were observed at the confocal laser scanning microscope (CLSM) and, the microdevices in contact, quantified. In parallel, these cells were studied under the scanning electron microscope (SEM) to assess the positions taken. Experiments stimulating these microdvices using ultrasonic pulses (ULTRASONIDO Sonic-Stimu Basic, Nu-Tek) are being performed on osteoblasts and visualized using Fluo-4AM (calcium dye) under confocal laser scanning microscope.

Results and Discussion

The microfabrication process in the clean room lead to the microparticles fabricated in FIG. 1A. As it can be seen in the image, discrete microparticles anchored to the substrate though a fragile stand with different sizes were obtained. The sizes of the microparticles were of 3.58 ± 0.04 µm x 3.56 ± 0.05 µm (small), 10.96 ± 0.05 µm x 6.68 ± 0.06 µm (intermediate) and 13.45 ± 0.64 µm x 19.27 ± 0.27 µm (big). After the hydrothermal growth, the microparticles were covered by ZnO NSs, which increased the size of the microdevice around 2 µm (FIG. 1B). The ZnO NSs thickness obtained was 14 to 17 nm. The whole process was highly reproducible and scalable.

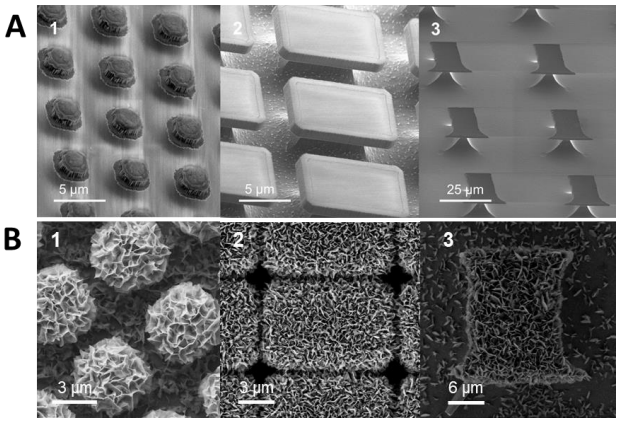

FIG. 1. Microdevice fabrication. A) SEM image of the microparticle array: microparticles with 3 x 3 μm^2 (1), 6 x 10 μm^2 (2) and 12 x 18 μm^2 (3) area. B) SEM images of the microdevices with the ZnO NSs piezoelectric layer.

After the peel-off step, the percentage of recovery of the microdevices was of 82% for the 3 x 3 μm^2 area and below a 30% for the intermediate (29%) and bigger size microdevices (26%). However, millions of microdevices are recovered from a single processed wafer [3].
After adding the microdevices into the cell culture in a 2:1 (small), 1:1 (intermediate) and 0.5:1 (big) proportion, cell viability remained above 85% for all of them, with respect to the control that was 96.5%.
The internalization of the microdevices, as expected, decreases as the size increases. Three positions are taken: internalized, NSs top down and NSs bottom up.
According to the results, these microdevices can be considered cytocompatible. They can also achieve a position outside the cell membrane with the ZnO NSs facing the membrane, an interesting position to trigger the VGCCs in the membrane.

Conclusions

Reproducible microdevices with different sizes and a piezoelectric layer of ZnO nanostructures were fabricated using microfabrication techniques and chemical synthesis. They were proved cytocompatible when cultured with Saos-2 cells. Also, the microdevices showed positions with respect to the cells from where the electrical response could be induced. This is necessary for our upcoming work, as ultrasound actuation is currently being validated with promising expectations.

Acknowledgments

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THE INFLUENCE OF THE SOLVENT ON MECHANICAL PROPERTIES OF GELATIN_ALGINATE HYDROGELS

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Introduction

Hydrogels are three-dimensional cross-linked polymeric materials that possess the ability to absorb a large amount of water or other polar solvents [1]. Young's Modulus, elongation at breaking point and tensile strength are some of the parameters that define the mechanical properties such as stiffness and elasticity of the hydrogel. Gelatin as a polar biopolymer dissolves quickly in hot water and creates gels due to cooling. Additionally, when chemically cross-linked, it gains better mechanical properties and reduction of degradation degrees [2]. Alginate salts, on the other hand, are converted into insoluble gels by cross-linking using divalent ions such as Ca^{2+} ions [3]. Mechanical properties of hydrogels composed of gelatin and alginate may also vary depending on the used solvent [4]. Thus the study aimed to test the mechanical properties of gelatin-sodium alginate hydrogels obtained using different solvents such as water and Dulbecco Modified Eagle Medium (DMEM), which is commonly used as the medium for the cell culture growth.

Materials and Methods

The experiment consisted of the preparation of 6% gelatin and 2% sodium alginate-based hydrogels prepared in water (G6_A2) and DMEM / F12K medium containing 10% FBS and a 1% mixture of antibiotics (identical to the 2D culture medium) (G6_A2_DMEM). Also, the materials were cross-linked by two different cross-linkers squaric acid (SQ) in 1% and dialdehyde starch (DAS) in 1% weight percent based on the dry weight of the protein. Finally, the hydrogels were immersed in a 1% calcium chloride solution for 10 min.
The mechanical properties were determined using the Shimadzu EZ testing machine (Shimadzu Corporation, Japan). The hydrogel was cut with a die into equal strips and was stretched. The Young's Modulus, tensile strength, and elongation at breaking point were determined. The obtained results were processed using TRAPEZIUMX software and Microsoft Excel.

Results and Discussion

The examined gelatin_alginate hydrogels presented distinct results depending on the solvent used for preparation. It was seen that the chemical cross-linkers also affected the tested materials. Hydrogels prepared in DMEM showed higher values of Young's Modulus, thus resulting in stiffer and more durable material than those prepared in H_2O. Moreover, the addition of DAS and SQ on G6_A2_DMEM hydrogel increased its mechanical parameters. On the other side, hydrogels prepared in H_2O were slightly lower than those in DMEM. However, the addition of DAS increased Young's Modulus values with a slightly decreased percentage of elongation at the breaking point compared to the base material G6_A2.

Conclusions

The mechanical properties tests confirmed the integrity and stability of the obtained biomaterials. The mechanical resistance is higher for materials prepared in the DMEM medium than using water. These results might be due to the interactions between medium components and gelatin-sodium alginate hydrogel. Also, results show a greater effect of DAS on materials prepared in water than those prepared in DMEM. This confirms that the use of cell culture medium on hydrogel preparation may significantly affect the materials' properties, and can be a potential solvent for more biocompatible biomaterial preparation. The obtained outcome presents important values for further characterization of the materials for biomedical research.

Acknowledgments

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DOUBLE-DOPED DIAMOND-LIKE CARBON COATINGS FOR POTENTIAL ORTHOPEDIC APPLICATIONS

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Introduction

An answer to the constantly growing demand for novel orthopaedic implants (related to increased life expectancy [1], number of traumatic injures [2], as well as a willingness to a quick return to professional sports and everyday activities) are, among the others, surface modifications of ready to use products. Diamond like carbon coatings (DLC) for such applications are the films that are not only under careful investigation of scientists devoted to biomedicine [3] but have already been introduced to the market solutions [4]. Via coatings doping, it is possible to tailor the performance properties of the implant. Using silver and copper as admixtures of carbon matrix induces the bactericidal or bacteriostatic effect due to the action of metals or their ions on microorganisms. Ag-DLC coatings effectively limit the growth of bacteria responsible for common hospital infections, such as *Staphylococcus aureus*. The 60% reduction of microorganisms viability can be obtained even within 3 hours of contact [5]. In the case of Cu doping, the bactericidal effect is lower for similar contents of admixture [6] but still can induce assumed properties. Coatings protecting from microbial colonization (also those involving Ag and Cu) can exert unwanted effect also on surrounding tissues. Such problems were already claimed for example by Sengstock et al. [7] in the case of silver ions and nanoparticles or by Zhang et al. [8] dealing with copper doped porous TiO_2.
The described research is trying to overcome the obstacles of DLC coatings with a bactericidal effect by introducing a low amount of silver and copper simultaneously.

Materials and Methods

All the examined coatings were deposited using magnetron sputtering in the argon atmosphere (process pressure equal to 0.5 Pa) on AISI 316 LVM austenitic steel. The samples were cylinders of 16 mm in diameter and 5 mm in height, that undergo mechanical polishing and degreasing in acetone.
The mechanical properties of the samples were estimated by means of Nano Indenter G200 (Agilent Technologies, USA) and multifunctional station UMT-2 (Bruker, USA).
The chemical examination involves usage of InVia (Renishaw) Raman microscope with backscatter geometry and AXIS Ultra DLD (Kratos Analytical) system for the X-ray photoelectron spectroscopy.
Biological evaluation of examined materials was covered by evaluation of microbial viability with use of BD Accuri C6 flow cytometer and model microorganisms *E. coli.* Additionally, the XTT examination was conducted for the sake of examination of osteoblasts proliferation.

Results and Discussion

Synthesis of Ag/Cu-DLC coatings allows the incorporation of no more than 10 % of dopants into the carbon matrix. The microhardness and Young Modulus of such materials were like for undoped coatings.
The admixtures were present in the coating in the partially oxidised state. The doping of DLC relates to increase of the fraction of sp^2 bonds. Observed was a shift of the G-band towards a higher wavenumber and an increase in FWMHG.
The biological examination allows the selection of the composition of coatings that ensure the highest bactericidal effect but no negative response of osteoblast cells.

Conclusions

It is possible to manufacture double-doped carbon coatings (containing silver and copper) by means of magnetron sputtering, which affect microbial colonization minimizing the negative effect on mammalian cells.

Acknowledgments

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FATTY ACIDS-STABILIZED NANOPARTICLES FOR PCL-BASED MAGNETIC FIBROUS NANOCOMPOSITES

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Introduction

Magnetic nanocomposites are promising materials for bone tissue engineering as they can support via magnetic field process of bone formation and remodelling [1]. The uniform dispersion of magnetic nanoparticles within polymer matrix is a critical issue in terms of psycho-chemical and biological properties of such composites. Herein, we proposed to use three saturated fatty acids of increasing length of carbon chain, namely caprylic acid (C8), palmitic acid (C16), and stearic acid (C18), as a surface-stabilizers of magnetic nanoparticles for magnetic fibrous polymer nanocomposites based on poly(ε-caprolactone) (PCL). Moreover, composites of three different wt.% of nanoparticles were prepared, i.e., 1, 2 and 5 wt%. The effect of type of stabilizer, as well as the content of magnetic nanoparticles on composites properties was investigated. For selected composite, containing 2 wt.% of caprylic acid-stabilized nanoparticles, the effect of different surface-treatment strategies (with NaOH or plasma) on biological response of SAOS-2 osteosarcoma cells was additionally determined.

Materials and Methods

Iron oxide-based nanoparticles were prepared via coprecipiation method and transferred into dichloromethane. Achieved nanoparticles were characterized by TEM and ATR-FTIR. Magnetic properties were measured using vibrating sample magnetometer. Fibrous nanocomposites were fabricated by electrospinning method under fixed conditions: infusion speed, needle diameter, distance between needle and collector. Fabricated nanocomposites were characterized by SEM (morphology), ATR-FTIR, TGA. Moreover, measurements of mechanical (tensile strength, Young's modulus) and magnetic properties was investigated. Two improve biological response of the composites, they were treated with NaOH of increasing concentration from 0.001 M to 0.3 M or with plasma for 30, 60 or 90s. *In vitro* studies were performed on SAOS-2 cells. The metabolic activity of cells was monitored on 1., 3., and 7. day of cultivation using MTT assay.

Results and Discussion

Synthesized nanoparticles were semi-spherical with diameter of 11 nm. Nanoparticles were superparamagnetic with magnetic saturation (M_S) of 63 emu/g Coating nanoparticles with saturated fatty acids provided their good stabilization in dichloromethane, which were major solvent for PCL. The presence of the stabilizer on the nanoparticles' surface was confirmed by ATR-FTIR (new bands at 11794, 2857, and 2956 cm^{-1}) and measurements of magnetic properties. In the ATR-FTIR spectra of stabilized nanoparticles new bands appeared at 11794, 2857, and 2956 cm^{-1}, which were assigned to fatty acids. In the case of magnetic properties, a decrease in magnetic saturation (M_S) associated with appearance of non-magnetic organic matter was observed; the longer carbon chain of fatty acid was, the more remarkable reduction in M_S was recorded. According to SEM, fibers in all nanocomposites and non-modified PCL had smooth and uniform morphology. Modification of polymer matrix with fatty-acid stabilized nanoparticles resulted in slight decrease in tensile strength and Young's modulus compared to non-modified PCL. The bright negative correlation between the length of carbon chain of fatty acid, as well as increasing content of nanoparticles was observed in the case of tensile strength at break, suggesting stronger interactions between caprylic acid-stabilized nanoparticles and PCL matrix, compared to palmitic acid- and stearic acid-coated nanoparticles (FIG. 1). Treatment of composites with NaOH resulted in deterioration of mechanical properties, which was not observed in the case of non-modified PCL. Saturation magnetization of fabricated composites correlated with the wt.% content of magnetic nanoparticles.

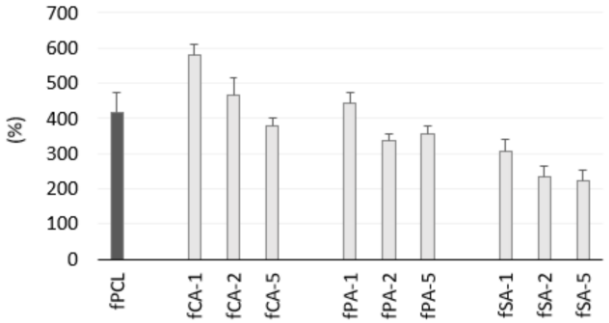

FIG. 1. Tensile strain at break of non-modified PCL and nanocomposites.

All fabricated nanocomposites were non-toxic towards SAOS-2 osteosarcoma cells. However, the significant effect of surface treatment was observed. Plasma treatment contributed to notably higher metabolic activity of cells on the first days of observations, while treatment with NaOH did not cause any changed, compared non-treated fibrous material. This indicates the facilitated cells attachment onto plasma-treated nanocomposites.

Conclusions

Stabilization of originally hydrophilic magnetic nanoparticles with saturated fatty acid is a simple way to provide their good distribution within hydrophobic PCL matrix. The use of shorter fatty acid contributes to stronger interactions between nanoparticles and polymer matrix. All materials were non-toxic, however, treatment their surface with plasma resulted in a significantly higher initial metabolic activity of SAOS-2 cells. Fabricated nanocomposite are promising materials for bone tissue engineering, however, further investigations with magnetic field need to be performed to evaluate the synergism of action between magnetic nanocomposites and remote magnetic field in terms of biological response.

Acknowledgments

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MULTIMATERIAL TUBE-LIKE SCAFFOLDS – 3D BIOPRINTING TECHNIQUE

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Introduction

The use of 3D bioprinting technology for medical applications is seen as a new upcoming standard, which will efficiently solve the donor-shortage issue for organ transplantations. Many different bioprinting techniques have been developed to design and produce scaffolds for tissue engineering and cell culture. Nevertheless, it is still very complicated to merge different materials and bioprinting techniques in one printing process to achieve a final multimaterial printout. [1-2] With designed and constructed system all above is possible and allows for printing with high temperature melting polymer and bioinks in one process that produces tube-like multimaterial elements that can be used in many branches of medicine where tubular constructs are needed, such as urology, cardiology or pulmonology.

Materials and Methods

Designed system is based on 3 printing heads. Two of which are for hydrogel, one of those is a bioink, and the third one is a FDM printing head for thermoplastic filament. All of them are incorporated in a one 3D printing machine. Printouts are manufactured on rotating stainless steel rod divided into three sections of different diameters. Smallest diameter plus width of the printout corresponds to bigger diameter is such a way that the printouts can be slipped on top of each other.
Hydrogel materials, for printing head 1 is mixture of sodium alginate and gelatine, printing temperature is 30^{o}C and for printing head 2 is mixture of sodium alginate and gelatine and living cells printing temperature is 34^{o}C. As material for printing head 3 there is a filament blend based on PLGA which printing temperature is 180^{o}C.
Materials are deposited on rotating stainless steel rod with three equal length sections of different outer diameters: 5.4, 5.7 and 6.0 mm. Printing head no. 1 deposits material on section 5.4 mm, printing head no. 2 on 5.7 mm and printing head no. 3 on 6.0 mm section. Final multilayer and multimaterial printout is obtained by sliding layer of bigger diameter on layer of smaller diameter. Each layer has 150 μm thickness to perfectly fit inside larger layer. Diameter of each section can be modified to reach the requirements of the final element dimensions.

Results and Discussion

Separate printouts produced on head no. 2 and 3 (FIG. 1).

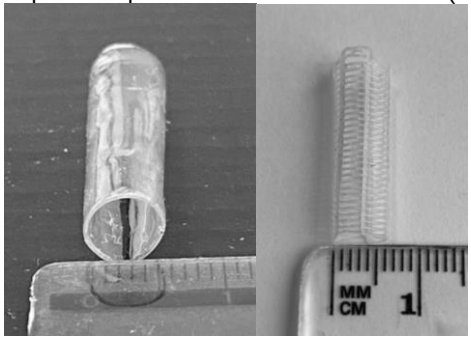

FIG. 1. Separate printouts produced on head no. 2 and 3.

Combined multimaterial printout is presented at FIG. 2.

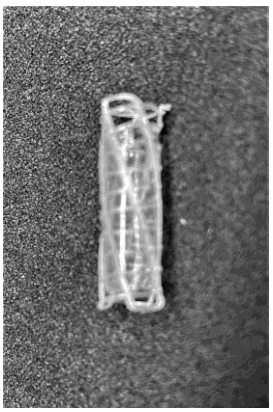

FIG. 2. Multimaterial printout, made out of 2 materials, hydrogel and high temperature melting polymer.

The printouts can be used as separate elements, but the main advantage of the system is that we can produce multimaterial tubular scaffold where we merge biomaterials with weak mechanical properties but good parameters for cell proliferation with materials of high mechanical properties but with weak cell adhesion. It allows for fabrication of well balanced scaffold for tissue engineering.

Conclusions

Fabrication of multimaterial 3D bioprintouts with the use of above mentioned technique may open new options of fabrication of tubular scaffolds. It allows for production of at least 2 layer tube like scaffolds, that can be made out of materials with different melting temperatures like bioinks and thermoplastic filaments. Such printouts might be used to solve problems of urology, cardiology and pulmonology.

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HIGH CARBON SILICON OXYCARDIBE BIOACTIVE MATERIALS

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Introduction

The so-called black glasses are polymer derived ceramics (PDC) of amorphous silica structure where part of oxygen atoms was replaced by carbon. This leads to creation of Si-C and C-C bonds (C-Si-O) which influence physical and chemical properties. One of the interesting features of amorphous silicon oxycarbide materials is its enhanced, compared to silica glass, bioactivity. This property can be further tuned by appropriate cationic modification using boron, copper or zinc ions, etc.

Materials and Methods

To create SiOC materials it was necessary to synthesize the preceramic polymers. To achieve this, the sol-gel method was used. Silicon containing alkoxides were used as reagents to obtain ladder-like polymers. Dried materials were then ceramized in flowing argon tubular furnace. The Kokubo test with Simulated Body Fluid was performed to examine the formation of apatite on the surface of prepared materials [1]. Spectroscopic studies (FT-IR, Raman, MAS NMR) were performed to assess the structure of obtained samples and to characterize the effects of Kokubo tests.

Results and Discussion

The subject of this work was to obtain high carbon silicon oxicarbides by the means of sol-gel process as bioactive materials. In order to achieve this, modified ladder-like silsesquioxanes were synthesized as preceramic materials. The amount of carbon was controlled by the introduction of methyl and phenyl groups with different ratio. This leads to the creation of Si-C bonds as well as C-C ones. Conducted structural studies allowed to control the synthesis process. Preceramic materials were characterized by the ladder-like structure with controlled ratio of Me/Ph groups.

Such prepared materials were then ceramized in a tubular furnace with flowing argon atmosphere. As it is known, only a small portion of carbon can be introduced into silica structure, while the rest is responsible for creating free carbon phase. This leads to creation a composite material C/SiOC. Therefore, by controlling the amount and distribution of C it is possible to adjust the physical and chemical properties of final materials. Detailed structural studies were conducted to characterize obtained materials. The analysis showed that studied samples, regardless of the Me/Ph ratio, exhibit similar SiOC structures, as expected.

To assess the bioactivity of received samples the Kokubo test was performed. The measurements revealed that the controlling of carbon content in SiOC composites influences the bioactivity.

Conclusions

Conducted structural and microstructural studies confirmed the presence of SiOC phase. The Kokubo test indicate that hight carbon black glasses have the potential of being bioactive materials.

Acknowledgments

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3D PRINTED COMPOSITE CNT/PLA SCAFFOLDS MODIFIED WITH HYDROXYAPATITE AND SILICON OXYCARBIDE

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Introduction

3D printing methods are broadly applied in various fields of science and industry including biomedical engineering. One of its main objectives is the development of materials used to support the reconstruction of damaged human tissues. Biodegradable polylactide (PLA) scaffolds are already applied to support bone regeneration, but their modifications are still demanded to ensure even higher efficiency of the healing process.

Materials and Methods

Composite filament based on PLA (BIOCOP PLLA Sn free, BioMatPol) with the addition of 2 wt% CNT (MWCNT, NanoAmor, USA) and the diameter of 1.75 mm was obtained using Composer 450 (3devo, Netherlands) extruder. Exemplary scaffolds with a specific shape, dimensions and microstructure were designed and prepared based on this material with the application of Fused Deposition Modelling (FDM) method using Creator PRO II (FlashForge, China) 3D printer. Their postprocessing included the surface modifications with commercially available hydroxyapatite (HAp, nanopowder <200 nm, ≥97%, Sigma-Aldrich, USA) and silicon oxycarbide (SiOC) powder synthesized according to the procedure described previously [1]. For this, the method of Electrophoretic Deposition (EPD) using custom-made equipment was applied. The microstructure of the samples was characterized by Scanning Electron Microscopy (SEM) with Phenom XL (ThermoFisher Scientific, USA).

Results and Discussion

3D virtual models were designed as cylinders with the diameter of 15.0 mm, the height of 3.0 mm and different porosities. Their microstructure consisted of the bars arranged in parallel and oriented at the angle of 90° between the layers. FDM process for the composite CNT/PLA filament was optimized to provide the appropriate reproduction of the virtual models. For this, the following parameters were applied: layer height of 0.2 mm, nozzle temperature of 200°C, bed temperature of 60°C and printing speed of 15 mm/s. Introduction of CNT into the PLA matrix resulted in increase of mechanical properties of the material and additionally provided its electrical conductivity necessary for the subsequent modification using EPD method.

The idea of surface modification of the scaffolds with hydroxyapatite or silicon oxycarbide is to improve their biological properties for the application as implants which support bone reconstruction [2,3]. For this, HAp or SiOC powder was added to ethanol and the resulting suspension was homogenized using ultrasonic tip. In the EPD system the specific scaffold was mounted as a main electrode and two stainless steel plates were used as counter electrodes. The process was performed under constant voltage (30 V) conditions. Solid and uniform coatings were obtained with the thickness proportional to the process time.

Conclusions

FDM provided the preparation of 3D CNT/PLA scaffolds with strictly predesigned architecture. Therefore, this method is an attractive alternative to the traditional ones applied to obtain materials for biomedical engineering. EPD provides the simple, fast and highly controllable preparation of the surface coatings. Therefore, it could be applied to modify the properties of the scaffolds by introduction of the specific additives, for example HAp or SiOC.

Acknowledgments

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THE CHITOSAN-BASED HYDROGELS CROSS-LINKED BY GLYOXAL

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Introduction

Currently, designing active substance delivery systems constitute one of the goals of novel materials development. Chitosan-based hydrogels are widely studied, however, their weak mechanical properties and stability provides the need to use cross-linkers. Glyoxal is chemical compound that contains two aldehyde groups. It is able to crosslink chitosan through Schiff's base formation between the free amino groups of chitosan and the aldehyde groups of glyoxal [1].
The aim of the study was to fabricate chitosan-glyoxal hydrogels and study their properties.

Materials and Methods

Chitosan (low molecular weight) and tannic acid were dissolved in 0.1M acetic acid at 2% concentration. Glyoxal was added to the chitosan solution in 5 and 10 w/w%. The mixture was slowly mixed on the magnetic stirrer for 1 min and left in room temperature for 15 min for gelation. As a results the gel-like form of hydrogels was obtained. Hydrogels were immersed in tannic acid solution (2, 5, 10, 20% concentration) for loading [2].
The morphology of the samples was studied using a scanning electron microscope (SEM) (LEO Electron Microscopy Ltd, England). Hydrogles were froze, lyophilized and covered by gold.
Dental pulp cells can be obtained from postnatal-, wisdom- and/or deciduous teeth, providing a non-invasive alternative (compared to e.g. bone marrow) to obtain osteoprogenitor cells. the hydrogels were placed in separate wells of 24-well culture plates and seeded with $2x10^4$ DPSC suspended in 2 mL of culture medium. The latter was composed of al-pha-minimum essential medium (αMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin 1% mixture). The cells were assessed for viability after 24 h culture on hydrogels or on TCP. For cell viability assay (CellTiter96Aqueous One Solution Cell Proliferation Assay; Promega), hydrogels were washed gently and carefully with PBS, followed by the addition of 0.4 mL/well of 10% MTS reagent in phenol-free alpha-MEM. The plates were incubated at 37°C until the apparent change of color from yellow to brownish. Then, the colored media were transferred to individual wells in 96-well plates and the absorbance was recorded at 492 nm using a plate reader (SpectraMax iD3, Molecular Devices, San Jose).

Results and Discussion

Hydrogel without tannic acid has a regular porous structure with open interconnected pores. Immersion in tannic acid solution results in the change in hydrogel's structure with closed pores (FIG. 1).

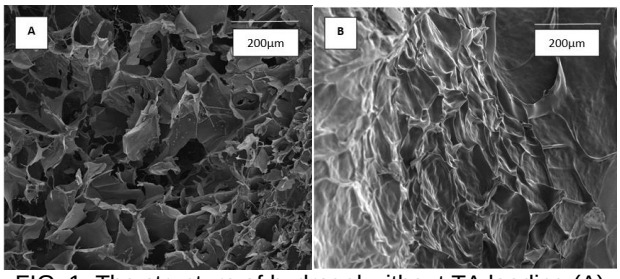

FIG. 1. The structure of hydrogel without TA loading (A) and hydrogel loaded by immersion in tannic acid solution at 20% concentration (B); magnification 500x.

Our research has shown that the tested hydrogels immersed in TA solution do not show a cytotoxic effect on hDPC (FIG. 2). A reduction in cell viability can be observed on hydrogels 2% and 20% in turn. The contents of 5% and 10% seem to stimulate the proliferation of cells grown on them. The hydrogel immersion in TA solution at concentrations 2, 5 and 10% did not affect the increase of cytotoxicity of material.

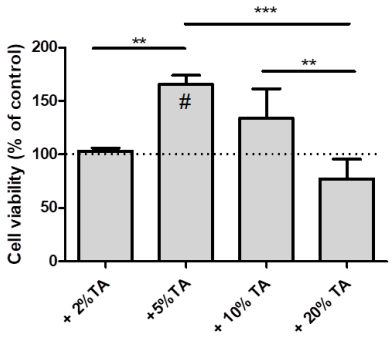

FIG. 2. hPDL cells viability on tested hydrogels after 24 h of culture. Results are expressed as % change in cell viability compared to control hydrogel. # indicates significant increased from control. Statistically significant differences (** $p \leq 0.05$; *** $p \leq 0.001$) within the groups.

The main application of hydrogels are drug delivery devices [3]. We confirmed that a simple method of hydrogels immersion in tannic acid is effective for TA incorporation into a hydrogel. Tannic acid may act as an anti-inflammatory factor and it has antimicrobial and anti-cancer properties.

Conclusions

Chitosan-based hydrogels were obtained successfully by the addition of glyoxal as a cross-linker. In addition, we have shown that polyphenols may be loaded to such hydrogels by simple hydrogel immersion in polyphenol solutions. Our studies suggest that obtained hydrogels may be used as phenolic acid delivery systems. Also, hydrogels did not show the cytotoxic effect. We believe the results of this work suggest that the obtained hydrogels may prove useful for several biomedical applications, such as wound dressings or bone-related treatment procedures.

Acknowledgments

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ALKYLATED DERIVATIVES OF HYALURONIC ACID AS KARTOGENIN CARRIERS

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Introduction

Cartilage diseases progress with age and result from making them a dominant problem in orthopedic surgery. Diseases of the skeletal system, particularly the joints, may have various origins. About 8% of the population is affected by degenerative joint disease, caused by deformation and damage to the cartilage due to excessive stresses. Another cause of diseases may be rheumatic diseases, to which every third person is exposed. In the case of very advanced lesions, alloplasty surgical procedures are used to replace the entire damaged joint with an endoprosthesis [1]. To reduce the need for surgical interventions, searching for new methods and preparations for inducing and supporting cartilage regeneration is essential. The main goal of the research is to improve delivery of kartogenin (KGN), as the only one synthetic substance that has shown the chondroprotective properties and potential of chondrogenic differentiation of mesenchymal stem cells [2,3]. Therefore, this biologically active substance can be used for the repair of cartilage tissue. The structure of KGN determines its hydrophobic properties. After exceeding a certain optimal value, hydrophobicity causes a decrease in the activity of the drug substance and its removal from the body too quickly, which is visible in the case of KGN. In order to reduce this negative effect various novel carriers were fabricated and examined.

Materials and Methods

The research premise was to receive structurally diverse polyanions (hyaluronic acid and chondroitin sulfate) and polycations (chitosan backbone). These polymers were modified with the use of carbon chains of various lengths and in varying ratios to obtain different grades. Hyaluronic acid was modified based on a methodology showed in the literature [4]. The scheme of this modification is shown on FIG. 1. By using polymer derivatives with different surface charges (cationic - positive and anionic - negative) it was allow to determine the influence of not only the chain length and degree of substitution, but also this charge on the physicochemical properties and bioavailability of the tested systems.
Properties of composed derivatives were investigated using spectroscopic methods and other advanced analytical methods. The obtained derivatives formed the basic components of the polyelectrolyte aggregates, which were used as KGN carriers. The final step was to assess the biological activity of the systems under study. Bioassays were subdivided into the cytotoxicity of the carriers.

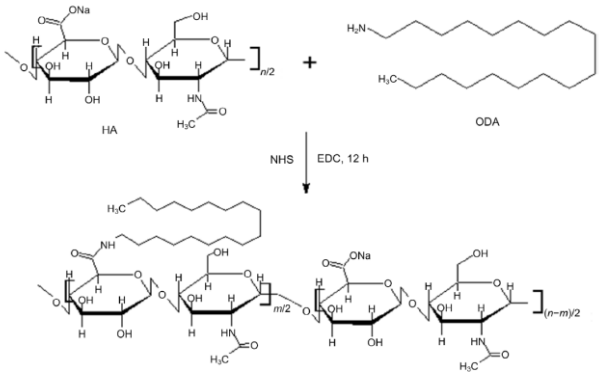

FIG. 1. Synthesis scheme of hyaluronic acid-octadecylamine (ODA) – C18. (NHS: 1-hydroxy-5-pyrrolidinedione; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)

Results and Discussion

The aim of the modification was to obtain a polymer with different alkyl chain lengths and different degrees of substitution (10% and 50%). Modifications of hyaluronic acid with alkyl chains were confirmed using the XPS technique. The spectra obtained show the increasing share of the C–C bond, which proves the correct course of the synthesis of substitution of alkyl chains to the polymer backbone. Then the obtained modifications were subjected to physicochemical characteristics. XPS spectra were used to calculate the degree of substitution.
The next step was to examine critical aggregation concentration (CAC) and the possibility of KGN encapsulation in each of the derivatives using fluorescence spectra.
The last stage of work was the preparation and characterization of polyelectrolyte aggregates with KGN. Physicochemical and biological tests of cytotoxicity were carried out on optimized systems. Toxicity analysis of modified hyaluronates showed that the addition of C12 alkyl chains with a degree of substitution of 10% reduces the viability of the sample cells above doses of 10 µg/mL of stimulant, while the addition of C18 chains also with a degree of substitution of 10% is highly toxic from the initial concentration of 0.5 µg/mL.

Conclusions

The resulting polymers are the promising carriers for kartogenin molecules. Such aggregates could have a potential application in cartilage tissue repair.

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ANALYSIS OF THE PHYSICOCHEMICAL PROPERTIES OF MODIFIED HIP ACETABULAR CUPS

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Introduction

Hip endoplasty is one of the most commonly used orthopaedic procedures to improve quality of life. The growing number of applications is linked to the increasing number of design and material solutions for hip prostheses and acetabular cups appearing on the medical market, which contributes to the dilemmas facing clinicians as to which solution to use. The most common material base for all solutions is titanium alloys, primarily Ti6Al4V [1]. In addition to some good physicochemical (mechanical, corrosion, etc.) or biological properties, there are also disadvantages, such as tribological or vanadium-related toxicological properties. These alloys, however, are susceptible to various surface modifications that allow the elimination of the disadvantages, as well as the additional improvement of others. The most common technological processes carried out to modify the surface properties of titanium alloys include: thermal and plasma spraying, anodic oxidation, ion implantation, vacuum CVD, PVD, electrophoresis, sol-gel method and laser treatment [2-4].

Materials and Methods

This study analyses the effect of surface modifications on the physicochemical properties of hip endoprosthesis acetabular cups made of Ti6Al4V alloy. Acetabular cups after four modifications were studied: (1) electropolished, (2) with a silicon doped carbon layer (Si-DLC), (3) with porous titanium and a Si-DLC layer, and (4) with porous titanium. The carbon layers were produced by RF PA CVD (radio frequency plasma-assisted chemical vapour deposition) and the porous titanium by APS (atmospheric plasma spray). All acetabular cups were manufactured using cavity methods and were sandblasted prior to the modification process. Layers of porous titanium were produced by applying titanium powder, with a grain size of 90-250 μm in a first step and then 25-90 μm in a second step. The fabrication of the carbon-silicon layers was preceded by a plasma etching process at up to 1400 V potential, followed by the actual fabrication of the carbon layers at 900-1000 V potential. The precursors used were a mixture of methane and CH4/HMDSO hexamethyldisiloxane. Macroscopic and microscopic analysis, as well as physicochemical property studies were performed using methods such as SEM-EDS, Raman spectroscopy and voltammetric ion release, among others.

Results and Discussion

Macroscopic and microscopic analysis carried out showed uniformity of the coatings in all cases. The few discolourations occurring in the case of porous titanium layers may be due to variations in the size of the powders used or the formation of conglomerates, which will consequently be subject to different rates of thermal decomposition and solidification processes in the plasma spraying device. The sites did not show differences in chemical composition. SEM-EDS analysis of the acetabular cups after electropolishing and with the Si-DLC layer alone showed few surface defects as remnants of the mechanical polishing treatment (mainly shallow scratches), indicating (in agreement with previous observations) that the thin carbon layer (approximately 300 nm) mimics the substrate. The shells in the Ti6Al4V_Ti and Ti6Al4V_Ti_SiDLC layer systems present a similar topography to each other, i.e. their surface is irregular and strongly developed. The chemical composition differs between the two modifications, the presence of peaks from silicon in the Ti6Al4V_Ti_SiDLC composite. In both cases, no peaks from the substrate, i.e. aluminium and vanadium, were found, indicating the high thickness and uniformity of the titanium coating. The Raman spectroscopy results showed a typical chemical structure of the fabricated Si-DLC coatings in agreement with literature data and no influence of the porous titanium interlayer. The results suggest a high reproducibility of the fabricated coatings on finished products in the form of hip acetabular cups. Tests on the surfaces of the titanium coatings showed the occurrence of the titanium oxide forms anatase and rutile on the surface.

Voltammetric tests allowed differences to be observed in the permeation of ions from the tested samples into physiological fluids, depending on the type of coating used. The analysis of the results obtained allowed to observe that in the case of Ti6Al4V and Ti6Al4V elements made of porous Ti, the permeation of titanium ions is more intense than that of vanadium, while for Ti6Al4V systems coated with SiDLC and Ti6Al4V systems coated with porous Ti and SiDLC, vanadium ions are released more intensively than those of titanium. These studies should be regarded as preliminary and need to be carried out on a larger scale.

Conclusions

The tests carried out on the hip joint acetabular cups supplied, modified in various ways, did not show, within the scope of the tests carried out, any negative impact of the modifications carried out on the functional properties of the implants and allows them to be used in the healthcare sector.

Acknowledgments

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NEW PROTECTIVE MATERIALS WITH A SKINCARE FUNCTION

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Introduction

The first case of COVID-19 appeared in Wuhan City on November 17, 2019, a previously unknown disease. It raised fear not only in Hubei province but also in the world [1]. In Poland, we have been struggling with it since March 20, 2020. It is a droplet-transmitted infectious disease. It attacks the respiratory tract and can cause severe complications such as pneumonia or respiratory failure. Several measures have been taken to fight this disease, i.e., restrictions on people staying in one room, a social distance of about 1.5 m, closing catering facilities or schools, and, most popular, wearing masks [2].
Disposable protective masks are one of the most important measures to prevent and help fight coronavirus infection. Since most people use disposable masks and throw them in the trash, the number of waste masks is constantly increasing.
There are over 9.1 billion tons of plastic in the world. According to scientists, by 2050, there will be over 13 billion tons of it, with used masks contributing to even more plastic. It will have a considerable impact not only on the animal environment but also on human life [1,3].
Wearing facial masks for a long time can cause the skin condition to deteriorate. The most common changes resulting from wearing it are acne vulgaris, skin irritation, and hyper-reactivity [3].

Materials and Methods

Our study focuses on developing a new prototype of protective masks needed in pandemic times. The novelty of the mask substitute is based on a polymeric matrix as opposed to the polypropylene interlining of which the standard mask is made. Its innovation is distinguished by the content of nettle and hemp oils. Such a composition will effectively eliminate dryness and irritation of the skin and reduce acne problems thanks to the properties of these active substances. The mask's composition will allow for its degradation, thus environmental protection.

Results and Discussion

Our work created a prototype mask based on biodegradable polymers (such as chitosan) with microparticles containing hemp and nettle oils. Their addition should eliminate skin problems resulting from wearing disposable protective masks. We used the encapsulation method to obtain microcapsules that release active substances. This solution has many advantages. It stabilizes the active substances and allows the ingredients to be delivered topically, controlled, and safe for the skin. Encapsulating the active substances in microparticles allowed their isolation from the environment.
The previously described problems of the COVID-19 pandemic, i.e., skin problems and environmental pollution caused by masks, could be eliminated by our mask prototype. The obtained polymeric matrix containing microparticles was fully characterized. The hemp and nettle oils have a positive influence on skin conditions. An important task was to check the water vapor permeability, as the mask should not impede breathing and be comfortable to wear. The last part of the research evaluates its effect on the skin. The essential features of the mask are non-irritation and acne-preventing properties.

Conclusions

The implemented project was to create a prototype of a protective mask with moisturizing and protective properties, preventing skin problems such as acne or irritation. Such a mask will be more comfortable to use. Its significant advantage is that it can be biodegradable. Animals will also benefit as they are surrounded by a lot of used plastic daily.

Acknowledgments

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CYTOTOXICITY OF SOL-GEL NANOLAYERS CONTAINING CARBON NANOMATERIALS

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Introduction

Investigations of various research groups are focused, particularly in recent years, on preventing of bacteria spreading through people touching various surfaces at sensitive areas (hospitals, clinics, public transport, shops, schools, etc.), thus reducing a potential epidemiological threat. This is an important problem especially in large human agglomerations and with people migrations (in EU millions of people travel from place to place every day). What is more, bacteria transferred through continents, brought with tourists and immigrants might be difficult to identify and treat. A potential solution of this problem is the application of antibacterial coatings, for instance obtained by sol-gel method, containing carbon nanoparticles (CNPs) [1,2].

The aim of this study was surface visualization and potential cytotoxicity investigation of nanolayers obtained by sol-gel method containing CNPs of three allotropic types. The rationale is that the antibacterial coatings should be durable and safe for the user.

Materials and Methods

Three types of carbon particles: nano-diamond (DND), reduced graphene (rGO) and carbon nanotubes (CNT) were used. Nanolayers on the stainless steel AISI 304 plates of 15 x 40 mm and thickness approx. 2 mm were prepared as follows: proper amount of nanoparticles were mixed with the sol of 3-(trimethoxysilyl)propyl methacrylate and tetraethoxysilane in isopropyl alcohol. Then the layers were obtained by: 1/ dip-coating (DC) and 2/ spin-coating (SC). Subsequently, after evaporation of isopropyl alcohol (RT, 15-30 min.), the samples were placed in a dryer at 150°C/30 min to complete the polymerization process. Characterization of hybrid layers was done by the Zeiss ultra plus scanning electron microscope to examine the quality of the layers.

Normal Human Primary Dermal Fibroblast (cell line ATCC-PCS-201-012) were used for cytotoxicity/ vibility testing using assays of XTT and Live/Dead™ Viability/ Cytotoxicity two-color (green-fluorescent calcein-AM and red-fluorescent ethidium homodimer-1). Study was done with fluorescent microplate reader (BioTek, Synergy HT) and fluorescent microscope (Zeiss, Axio Observer). Image analysis was performed using a custom-built workflow in Matlab 2019a (The MathWorks, Inc.), the human fibroblasts cells properties (live/dead) on the surfaces in the fluorescence images were analyzed. The ratio of green-stained cell areas (live) to red stained cell areas (dead) was assessed. Two parameters were used because of the aggregation of cells into colonies (area and total cell count). Other parameters as perimeter, roundness, length and width were determined as well. The steps were conducted within program script.

Results and Discussion

There are some challenges in preparing sol-gel nanolayers containing nanoparticles mainly due to incomplete particles dispersion and agglomeration. Two coating methods (dip-coating and spin-coating) have been used in parallel, whereas these methods provide usually different layer quality.

In the case of DND, the initial dispersion was already ineffective. The layers contain predominantly clumps (both deposition methods). We assume, that DND are be firmly fixed in the layer. rGO initial failed to disperse; the layers contain only clumps (both deposition methods). The clusters of graphene were evidently very poorly wetted in the sol. The particles were spread onto the surface of the layers, where they appear as separate particles in the final stage of layer preparation. The best results were obtained with carbon nanotubes CNT. Most of the nanotube aggregates were dispersed well during the initial dispersion. Both deposition methods gave similar results and are well applicable.

The obtained results of the XTT test are presented in the FIG. 1. According to ISO 10993-5, a reduction in cell viability below 70% means a cytotoxic effect, thus the tested materials did not show cytotoxic activity.

Life/Dead assay results showed that the layers containing DND and rGO somewhat restrict proliferation of cells, and CNT containing layers might be even harmful to cells.

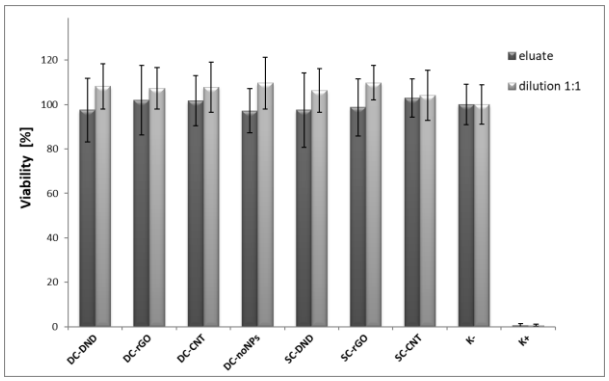

FIG. 1. Viability of human fibroblasts after 48 h incubation in the presence of eluates of investigated samples, K^- culture medium, K^+ 0.01% Triton X100 solution.

Conclusions

The production method: dip-coating and spin-coating does influence on the quality of the coating on steel plates. All obtained sol-gel nanolayers containing nano-diamonds (DND), graphene (rGO) and carbon nanotubes (CNT) are not toxic on human fibroblast cells. Nevertheless, the sol-gel layers containing carbon nanoparticles do not support proliferation of cells. This does not restrict potential application of sol gel coatings with carbon nanoparticles on surfaces in sensitive public areas.

Acknowledgments

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COMPARISON OF TWO POLYMER BIOMATERIALS MODIFIED WITH METAL IONS IN THE CONTEXT OF WOUND HEALING

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Introduction

Chronic wounds remain a serious therapeutic challenge. The lack of a universal method for effective treatment prompts scientists to develop new, effective therapeutic solutions [1]. Currently, high hopes are placed in polymeric biomaterials enriched with metal ions. Due to beneficial antimicrobial activity, copper ions are of great scientific interest. In turn, dressings enriched with calcium ions support homeostasis as well as proliferation of fibroblasts and keratinocytes, accelerating skin regeneration processes [2,3]. The goal of this study is to compare structural, physicochemical, and biological properties of curdlan- based biomaterials improved with copper or calcium ions in the context of wound healing.

Materials and Methods

The curdlan biomaterials were made by ion-exchanging dialysis method against copper or calcium ions. Briefly, curdlan powder was dissolved in 0.3 M NaOH solution and such mixture was placed in cylindrical molds. Then, 2% solution of copper chloride ($CuCl_2$) or 2% solution of calcium chloride ($CaCl_2$) was added to the polymer solutions. After that, cross-linked biomaterials with a hydrogel structure were subjected to two-stage freezing and freeze-drying. The structural properties of biomaterials were analyzed using SEM and EDS. The ability of biomaterials to release metal ions to culture medium was evaluated via commercially available spectrophotometric assays. The absorbent ability of the biomaterials was estimated using simulated wound fluid (SWF). Water vapor transmission test was also performed in order to evaluate the gas permeability of the biomaterials. Next, biomaterials enriched with copper ions were assessed for antimicrobial properties using disc diffusion method against Gram + and Gram – bacteria and subjected to cytotoxicity evaluation towards normal human fibroblasts (BJ cell line). In turn, biomaterials improved with calcium ions was surrendered to evaluation of viability and proliferation of BJ cells.

Results and Discussion

The scanning electron microscope (SEM) images showed that both types of biomaterials possessed porous structure. Moreover, it was indicated that surfaces of tested curdlan-based biomaterials were covered with precipitates, which – based on obtained EDS spectra – were composed of cooper and chlorine (after dialysis against $CuCl_2$) or calcium and chlorine (after dialysis against $CaCl_2$). The measurement of differences of biomaterial weights (before and after their incubation in simulated wound fluid (SWF)) showed that all tested biomaterials had good ability to absorb liquid. Moreover, it was shown that both types of curdlan-based biomaterials were permeable to water vapor with water vapor transmission rate (WVTR) values close to 2000 g/m^2/day. Afterwards, it was proved that the tested biomaterials had the ability to release calcium or copper ions to the culture medium. As mentioned previously, copper ions have antimicrobial properties, while calcium ions are of key importance in the wound healing process. Thus, an initial antibacterial test (disc diffusion method) demonstrated that biomaterials enriched with $CuCl_2$ had ability to inhibit *E. coli* and *S. aureus* growth. Unfortunately, at the same time, they were highly toxic towards BJ cells. In turn, biomaterials enriched with calcium ions were not only non-toxic towards BJ cells but also enhanced their viability. Moreover, it was revealed that these biomaterials promoted proliferation of skin fibroblasts. The main features of two types of biomaterials were summarized in FIG. 1. More details about obtained results can be found in [4,5].

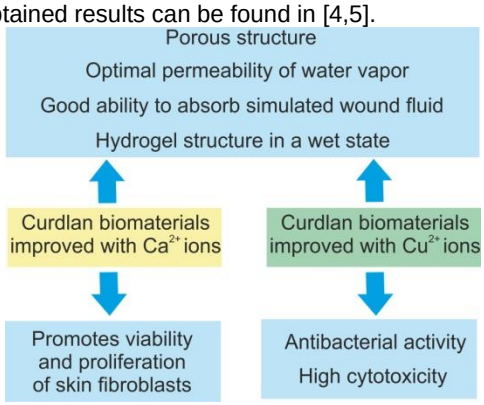

FIG. 1. The main properties of curdlan biomaterials modified with calcium ions (Ca^{2+} ions) or copper ions (Cu^{2+} ions). The biomaterials were characterized for potential use as wound dressings.

Conclusions

In summary, curdlan-based biomaterials made by ion-exchanging dialysis against calcium ions had a porous structure, released calcium ions, possessed good ability to absorb SWF, and showed the transmission of water vapor at an appropriate level. Moreover, the extracts obtained from these biomaterials were non-toxic and supported proliferation of skin fibroblasts *in vitro*. Received biomaterials modified by cooper ions also possessed porous structure, good ability to absorb SWF, and had capacity to release copper ions to the aqueous environment. These biomaterials had also antibacterial properties and enabled water vapor transmission on optimal level. However, compared to the calcium ion-modified biomaterial, the produced biomaterials showed cytotoxicity to human normal skin fibroblasts. Taking into account aforementioned results, it is clear that the curdlan-based biomaterials enriched with calcium ions are a good candidate as a wound dressing.

Acknowledgments

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BIOMATERIALS BASED ON THE EXTRACELLULAR MATRIX AS THE FUTURE OF TISSUE ENGINEERING. IS THERE AN UPPER LIMIT ON THE dECM CONTENT IN BIOPRINTING TISSUE?

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Introduction

Three-dimensional (3D) bioprinting is one of the most promising and fastest developing technologies for the production of bionic tissue structures as well as whole organs with a vascular system. For example, the world's first print of a bionic pancreas with a complete vascular system. The key element of bioprinting is the use of appropriate biomaterials that ensure the viability and functionality of the cells used in this process. One of the most popular biomaterials are those based on the extracellular matrix (dECM) derived from natural tissues. They are characterized by a natural composition and complex biochemical properties. As a result, it is possible to even more accurately reproduce the native environment for cells. However, what is the most optimal dECM concentration in the biotope? Does more mean better? The aim of this study was to find answers to these questions.

Materials and Methods

Two types of biomaterials were prepared for the study - bioink precursors, which were made on the basis of extracellular matrix derived from pancreatic organs subjected to the decellulatization process. Bioinks precursors were prepared in a standard, commonly described manner using enzymatic methods. In addition, however, crude dECM was added to each of the hydrogels. The final bioink contained from 2% to 16% dECM. In conclusion, the variants with 16%, 14%, 13%, 11%, 10%, 8%, 5% and 2% of dECM were analyzed. Constructs for the assessment of cell viability, cell functionality were printed from the bioinks prepared in this way, which were performed using a glucose stimulation test (GSIS test conducted for 21 days), cytotoxicity (MTT test) and histological evaluation. All tests were performed on beta cells (INS-1E).

Results and Discussion

The evaluation of bioinks precursors began with their bioprinting capabilities. In the case of all 8 variants, prepared according to the same protocol, it was possible to bioprint homogeneous, stable constructs. During the bio-printing process, it was observed that parameters such as: temperature, printing speed and pressure depend on the final dECM content in the bioink.
In the case of increasing dECM content, the necessity to use higher pressures and a higher temperature of the bio-ink itself was demonstrated. Correspondingly, the pressure was 6-48kPa, and the temperature was 16-25°C. Also, to obtain a better print resolution, the printing speed was changed and ranged from 5-20 mm/s.
The next step was to evaluate the cytotoxicity of the analyzed bioinks.
First, the cytotoxicity of dECM itself was tested (without any physicochemical treatment). The results of these analyzes showed complete safety of the analyzed product. Therefore, the next step was to prepare the bioink according to the assumed parameters and to print the constructs for the proper MTT tests. The test results showed differentiated results depending on the tested variant. The least favourable results were obtained for variants above 13% dECM content. On the other hand, the most positive results for the viability were shown for bioinks with a dECM content of up to 11%. The obtained results showed a significantly higher percentage of viability depending on the tested variant, the difference was even 5% lower survival of INS-1E cells. On the other hand, the addition of dECM in the range of 8-11% showed even 25% higher cell growth than in the group of untreated cells.
Confirmation of the results for the MTT test was obtained during the GSIS analysis. The functionality of beta cells was highest in the case of the bioink containing up to 11% dECM. The beta cell functionality in these variants was the most stable and was maintained through 21 experiments. However, the best results were obtained for the variant of 5% and 8% dECM content.
Histological analysis confirmed the advantage of bioinks up to 11%, showing a 3-fold increase in the insulin signal in these bioinks. In these analyzes, the 5% and 8% variants also showed a stronger signal for insulin secretion.
The obtained results clearly confirm that the most optimal content of dECM in bioprinted constructs (with beta cells) should vary from 5 to 10% of its content in the final bioink. These results are confirmed by a cascade of biological research.

Conclusions

The experiments carried out in this study prove that the appropriate concentration of dECM, which is the natural environment for a cell, must be properly validated in the case of tissue engineering and selected for a given type of cell. In the case of beta cells, it should not exceed a total of 10% of the dECM. It should be remembered that the ECM serves not only as physical protection for cells in the native environment, but also helps in various biological functions. Therefore, the more its concentration is crucial for maintaining appropriate living conditions.

Acknowledgments

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

Conflicts of Interest

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

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INFLUENCE OF THE SONICATION PROCESS OF BIOINKS PRECURSORS ON THE QUALITY OF BIODEGRADABLE STRUCTURES – EVALUATION OF PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

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Introduction
Hydrogels consisting of the extracellular matrix (ECM) are used in modern regenerative engineering to create bionic constructs. For the production of dECM-based hydrogels, enzymatic methods, special extraction buffers and dialysis procedures are most often used, which may affect the structure and function of proteins obtained after the decellularization process. This study aimed to determine the biological properties of bioink precursor based on dECM prepared by sonication.

Materials and Methods
The study involved a comparison of bioink prepared with the enzymatic (BE) method and the sonication method (BS). The pig pancreas were the basis for obtaining dECM. From 18 to 20 kg of organs were used for one dECM production cycle. The following tests were performed on the bioinks precursor prepared in this way: cytotoxicity assessment performed with the MTT test, assessment of beta-cell functionality (INS-1E) in the glucose stimulation test (GSIS test) as well as a histological and rheological assessment after completion of the stimulation tests. All biological tests were carried out on INS-1E cells and were performed with a minimum of 3 biological replications.

Results and Discussion
The characterization of the bioink began with the assessment of the possibility of bioprinting stable constructs. An acceptable result was obtained for both bioinks precursors. The constructs were stable and easily moved from the bioprinter's table. There was no difference in the pressures used during printing. Both bioinks required pressure of up to 10 kPa. The situation was different in the case of the bioink temperature. The bioink precursor prepared with the enzymatic method (BE) was best printed at a temperature below 20°C, whereas after sonication, the bioink (BS) required increasing the temperature above 20°C. To ensure a satisfactory print resolution, in the case of BS bioink precursor, the speed could not exceed 10 mm/s. However, in the case of the BE bioink precursor - it was higher by 2-3 mm/s.
Prepared constructs were then tested for cytotoxicity. The tests were carried out for 72 h and in the final stage of the analysis, it was shown that the survival of cells in the BS bioink precursor was 2.5-fold higher than in the BE bioink. It is an important parameter in further biological analyzes, which has also been translated into the study of functionality.
The GSIS test showed an almost 2-fold higher concentration of insulin secreted per glucose stimulus. Despite the noticeable differences in the functioning of beta cells in histological imaging, no differences in staining for insulin were shown. A comparable number of cells was stained in both the BE and BS variants.
The rheological analysis showed comparable values of the G 'and G "modules for the sonication and enzymatic bioink variants. It can be noticed, however, that the flow point [PP] for the BS version is slightly lower. PP) compared to the same constructs prepared based on enzymatic dECM.
The presented results show a significant advantage of the dECM-based bioink precursor prepared with the sonication method over the commonly used enzymatic method. Such bioinks is less cytotoxic, cells have longer functionality and the structure itself is stable, although in the case of BS it is more sensitive to mechanical damage. Therefore, depending on the further application, it is worth considering both preparation methods.

Conclusions
Biomaterials used in modern tissue engineering can find applications for bioprinting bionic tissues and organs with living cells, and as scaffolds for their subsequent colonization. Depending on the application, it is worth considering the method of their preparation, taking into account, for example, their cytotoxicity.

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

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

WHY IS THE CELL ELASTICITY IMPORTANT MARKER? – ON THE EXAMPLE OF HUVEC CELLS EXPOSED TO PAMAM DENDRIMERS

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Introduction

A cell is the smallest building block of our body that affects its entire functioning. Cells are most often analyzed in terms of their biological parameters, however, their physical properties should also be considered. One of the physical parameters describing cells is elasticity, i.e. the ability of a material (here a cell) to return to its original shape after removing external forces. Elasticity depends on the cells function and their location [1,2].

This work comprise the presentation of force spectroscopy methodology for the study of the mechanical properties of endothelial cells (ECs). The aim of this study is to assess the effect of polyamidoamine (PAMAM) dendrimers of 2nd, 4th and 7th generation on human umbilical vein endothelial cells (HUVEC).

Materials and Methods

Primary endothelial cells were exposed to PAMAM dendrimers for 24 h, using concentrations reducing cellular viability to the levels of 90%, 75% and 50%. We assumed, that changes in mechanical properties reflect toxicity of PAMAM dendrimers.

The mechanical properties were investigated using atomic force spectroscopy (AFS) technique with the use of two approaches for measuring cell elasticity: global, where the tests were performed using a micrometer-hemispherical probe, and local, where a nanometer-sized probe was used.

A presence of nanostructures on the cell surface and inside the cell was examined using scanning and transmission electron microscopes. Additionally, an alteration of actin fibres of the cellular cytoskeleton was examined by fluorescence microscopy.

Results and Discussion

For the sharp probe, a reduction in the elasticity modulus was observed in comparison to untreated control cells, that is related to the depolymerization of the cytoskeleton and the processes leading to cell apoptosis. In the case of the hemispherical probe, cell softening was also observed in comparison to control cells, but with increasing PAMAM concentrations, the modulus of elasticity increases. It is related to the sensing of numerous intracellular vesicles with the use of this probe e.g. endosomal and empty plasmalemmal which can also alter cell elasticity. The presence of external and intracellular vesicles was confirmed by scanning and transmission electron microscopy. The relationship between the elasticity of HUVEC cells exposed to PAMAM dendrimers of selected generations and their toxic effects was presented herein for the first time. In the transmission electron microscopy images of the cells exposed to PAMAM dendrimers, we have also observed distinctive vesicles with regular multilayer arranged structure (FIG. 1). The part of the presented results were already published in the paper [3].

Conclusions

In our work we confirmed nanotoxicity of PAMAM dendrimers on endothelial cells manifested by changes in cell elasticity. Cell softening was detected as the concentration of PAMAM dendrimers increased. As regard to cells exposed to PAMAM G2 and G4 dendrimers, the effect is dose-dependent. Many intra and extracellular vesicles formation both on the cell surface and inside the HUVEC cells incubated with PAMAM dendrimers was observed by scanning and transmission electron microscopy.

We postulate that elasticity modulus can be used as an effective indicator of physiological cell state and evaluation of nanotoxicity.

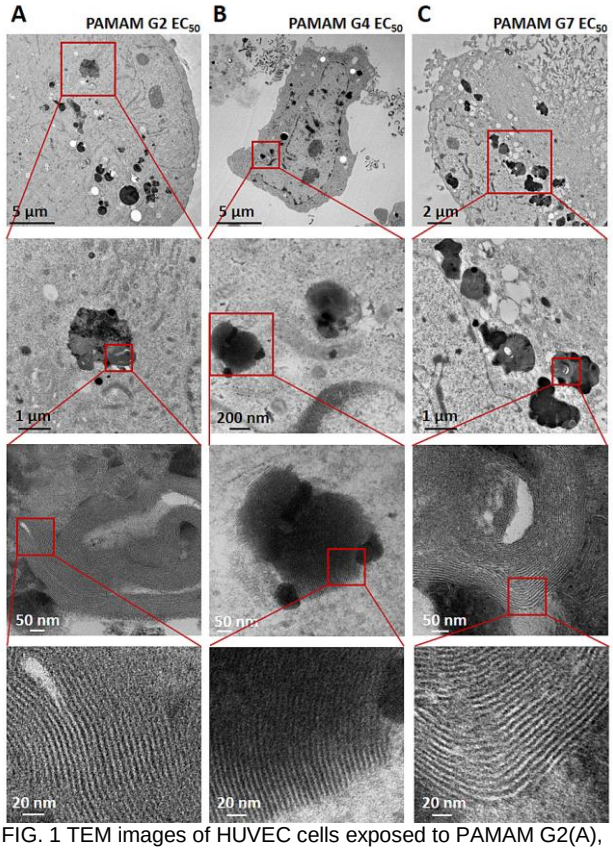

FIG. 1 TEM images of HUVEC cells exposed to PAMAM G2(A), G4(B), G7(C) at EC_{50} concentrations (defined as 50% of cellular viability) at different magnifications. The consecutive bottom images present the enlarged areas from marked red squares. In the lowest bottom images, the regular multi-layers structures are presented.

Acknowledgments

This work is financed by The National Science Centre, project title: "The influence of selected nanoparticles on the elastic properties of endothelial cells evaluated using atomic force microscopy," agreement no. 2017/26/D/ST4/00918.

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STUDY OF *STAPHYLOCOCCUS AUREUS* ADHESION ON CoCr ALLOY WITH ZnO-ZrO_2 LAYER

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Introduction

In order to restore the proper functioning of the stomatognathic system, dental prosthetics provides a number of possibilities that are individually adapted to a specific patient. One of the best solutions to compensate for missing teeth for patients without implantable conditions is the use of a partial skeletal denture [1]. The use of a skeletal denture also significantly reduces treatment costs compared to implants. The introduction of 3D printing technology meant that this method was also used in the production of prosthetic components [2]. Although prostheses made with this technology are better suited to the patient's anatomical features, surface preparation is still challenging, if only because of their complicated shape, which provides an ideal habitat for bacteria and fungi. The innovative ALD method enables the protection of the prosthesis surface against its interaction with the oral cavity by applying an antibacterial and antifungal layer with appropriate parameters physicochemical properties [3-5].

Materials and Methods

CoCr alloy samples made in additive technology - 3D printing by SLS method, after electrochemical polishing with parameters allowing for surface roughness Ra <0.3 µm were selected for the tests. The shapes of the samples were discs with a diameter of d = 13 mm and thickness = 3 mm. The chemical composition and mechanical properties were in accordance with the ISO 22674 standard. The surface layer was ZnO_2 and ZrO_2 in the proportions 10:90, 50:50 and 90:10 applied with the ALD method at the temperature of 200°C and 300°C. Bacterial adhesion tests were carried out using the *Staphylococcus aureus* bacterial strain (ATCC 25923). Before testing, the inoculated bacteria were incubated for 18 hours at 37°C in 30 ml of Bacto ™ Tryptic Soy Broth (TSB) (Becton Dickinson). Then, the test samples were placed in 24-well plates. 1 ml of bacterial suspension (~ 5·106 CFU·cm^{-3}) was added to their surfaces and the samples were incubated at 37°C for 4 hours. Then the culture medium was withdrawn and the samples were washed with sterile PBS solution. To remove adhering bacteria, the samples were placed in new 24-well plates and flooded with 1 ml of 0.25% trypsin in water. The samples were vigorously shaken in the solution for 30 s. Then, 100 µl of the solution of bacteria and trypsin were taken, and then mixed with 0.9% NaCl in concentrations of 1:1000, 1:10,000 and 1:100,000. 100 µl of the prepared solutions were spread on the plates agaric. The agar plates were incubated at 37°C for 18 hours. Live colonies of bacteria were then counted.

Results and Discussion

The ZnO-ZrO_2 layer favorably influences the bacteriostaticity of the CoCr alloy substrate. Regardless of the concentration of elements (Zn, Zr) in the surface layer, a reduction in the amount of bacteria was found compared to the surface of the substrate. The temperature of the ALD application process also influences on the presence of bacteria – FIG. 1.

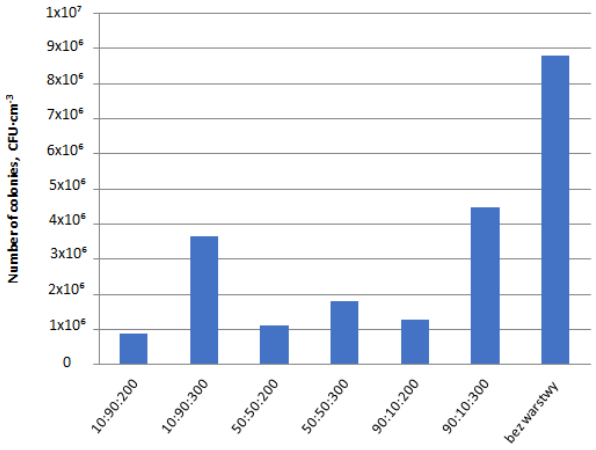

FIG. 1. Staphylococcus aureus (ATCC 25923) adhesion on layer ZnO-ZrO_2 (number of bacterial colonies after 4 h of incubation at 37°C).

Lowering the process temperature from the value of T = 300°C to the value of T = 200°C resulted in a reduction in the number of bacteria, regardless of the concentration of the elements constituting the layer. This may be due to the absence of oxides of elements derived from the substrate, i.e. Co, Cr, Mo, formed on the surface at elevated temperatures.

Conclusions

The research carried out for the ZnO-ZrO_2 layer applied by the ALD method in the process parameters proposed by the authors on the CoCr substrate clearly showed that the surface layer, regardless of the Zn and Zr concentration, has antibacterial properties and constitutes an effective barrier for deposition of the S. Aureus bacteria on the surface of the dental skeletal prosthesis.

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THE DESIGN OF AN IMPLANTABLE DELIVERY SYSTEM WITH ARIPIPRAZOLE

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Introduction

Aripiprazole (ARP) is an atypical neuroleptic used in the therapy of schizophrenia. The lack of optimal adherence to an oral therapy regime creates a serious challenge [1]. Therefore, long-acting injections with ARP are still being developed. However, the administration of currently used liquid formulations with ARP is associated with pain, no possibility of removing, and often difficult outpatient care. Moreover, the formulation of proposed biodegradable nano- and micro- formulations based on poly(lactide-co-glycolide) generates difficulties with the use of an organic solvent and a different effectiveness of ARP loading (43.2% - 80.0%) [2, 3] compared to rods formulated by hot melt extrusion (HME) (94.3 ± 1.4%) [4].
The study aimed to design and characterize the implantable delivery systems with ARP in the form of rods manufactured by HME from three terpolymers based on D,L-lactide (D,L-LA) or L-lactide (L-LA), glycolide (GA), and trimethylene carbonate (TMC) with different molecular weight (M_n).

Materials and Methods

Poly(D,L-lactide-co-glycolide-co-trimethylene carbonate) with M_n of 21 kDa ($T_{D,L}$ 21), poly(L-lactide-co-glycolide-co-trimethylene carbonate) with M_n of 59 kDa (T_L 59) and with M_n of 77 kDa (T_L 77) were synthesized in bulk via the ring-opening polymerization of D,L-LA or L-LA, GA, and TMC (HUIZHOU Foryou Medical Devices Co., Ltd., China) at 120°C for 72 hours in the presence of zirconium (IV) acetylacetonate (the ratios of initiator to monomers: 1:600, 1:1000, and 1:1200). The rods with ARP (Zhejiang Huahai Pharmaceutical Co., Ltd., Linhai City, China) (10% w/w) were formulated at 105°C by HME [5]. NMR, DSC, FTIR, and SEM were applied to characterize the terpolymers and rods with ARP [5].

Results and Discussion

The HME did not influence the composition of the terpolymers. First heating runs for raw terpolymers revealed a lack of significant thermal events pointing out an amorphous character of polymers. The analysis of DSC curves of the rods with ARP showed the endothermic events coming from crystalline ARP [4]. The second heating run revealed the decrease in glass transition temperature (T_g) after ARP introduction, which may suggest the plastification and consequently the increase in bioavailability (TABLE 1). The HME resulted in a decrease of M_n in the range of 13% - 45% (TABLE 1). These effects may be caused by thermal degradation and random chain scission as a consequence of oxygen or water presence [6]. However, a decrease to ~70.0% was observed by other authors [5].

TABLE 1. Parameters characterizing raw terpolymers and rods with ARP. F_{LL}, F_{GG}, and F_{TMC} - molar percentage of LA, GA, and carbonate units, respectively; T_m - melting temperature; ΔH - melting enthalpy; T_g - glass transition temperature; M_n - molecular weight; D - molecular weight distribution; ND - non-detected.

	$T_{D,L}$ 21		T_L 59		T_L 77	
Sample	**Raw**	**Rod-ARP**	**Raw**	**Rod-ARP**	**Raw**	**Rod-ARP**
F_{LL} [mol%]	63.40	63.60	66.50	64.30	57.10	56.20
F_{GG} [mol%]	14.70	15.90	16.60	18.60	18.30	18.30
F_{TMC} [mol%]	19.90	20.60	16.90	17.10	24.60	25.60
T_m [°C]	ND	128.70	ND	133.40	ND	133.60
ΔH [J/g]	ND	8.40	ND	5.20	ND	3.40
T_g [°C]	36.30	33.70	46.70	41.80	39.90	37.40
M_n [kDa]	21.40	18.70	59.40	55.20	76.80	43.90
D	1.97	2.00	1.95	2.01	2.00	1.93

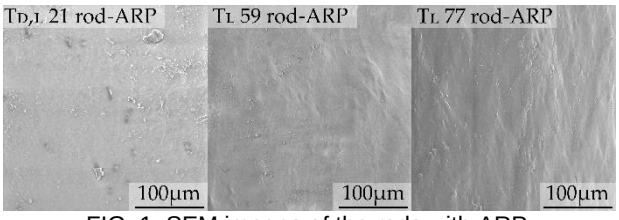

FIG. 1. SEM images of the rods with ARP.

The surface of the rods showed the solid character observed for amorphous materials. The HME process did not result in unfavorable morphological features such as cracks, microcavities, or slits (FIG. 1).

Conclusions

The HME allowed the formulation of the rods with ARP with properties qualifying them for further research.

Acknowledgments

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THE INFLUENCE OF TERPOLYMER PROPERTIES ON ARIPIPRAZOLE RELEASE

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Introduction

Currently, in the treatment of mental disorders, parenteral biodegradable formulations with a prolonged release of atypical neuroleptic are preferred [1].
This study aimed to determine the role of the structural, and thermal properties of poly(L-lactide-co-glycolide-co-trimethylene carbonate) (P(L-LA:GA:TMC)) in the release of aripiprazole (ARP) from the rods formulated by hot melt extrusion (HME) and electron beam (EB) irradiation.

Materials and Methods

The rods with 10% w/w of ARP (Zhejiang Huahai Pharmaceutical Co., Ltd., Linhai City, China) based on P(L-LA:GA:TMC) were formulated by HME (P(L-LA:GA:TMC) rod-ARP) and EB irradiation (EB-(P(L-LA:GA:TMC) rod-ARP)) [2, 3]. Rod degradation was carried out in a PBS solution. HPLC; NMR; DSC; molecular weight (M_n); water uptake (*WU*); weight loss (*WL*); and SEM were done according to the previous methodology [3].

Results and Discussion

ARP was released in a tri-phasic model with a lag phase (FIG. 1). The release profiles reflected the structural and thermal properties. No unfavorable changes in terpolymer content were observed. A decrease in the glass transition temperature (T_g) and the M_n, and an increase in the *WU* and the *WL* were revealed (TABLE 1).

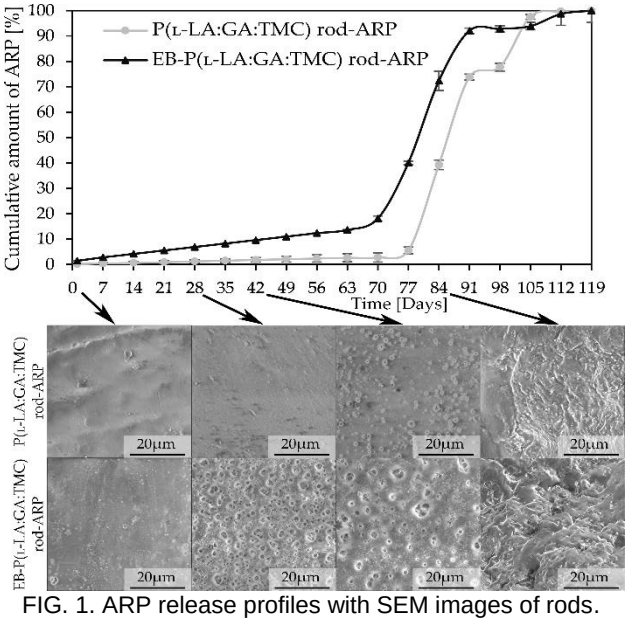

FIG. 1. ARP release profiles with SEM images of rods. EB irradiation induced earlier pore formation, more intense *WU* and *WL* changes, and a faster release (FIG. 1, TABLE 1).

TABLE 1. Parameters characterizing the rods. F_{LL}, F_{GG}, and F_{TMC} - molar percentage of LA, GA, and carbonate units, respectively; T_m - melting temperature; ΔH - melting enthalpy; T_g - glass transition temperature; M_n - molecular weight; *D* - molecular weight distribution; *WU* - water uptake; *WL* - weight loss; ND - non-detected.

P(L-LA:GA:TMC) rod-ARP

Time [Days]	0	28	42	84
F_{LL} **[mol%]**	58.30	58.10	58.10	54.00
F_{GG} **[mol%]**	18.20	17.90	17.70	12.90
F_{TMC} **[mol%]**	23.50	24.00	24.20	33.10
T_m **[°C]**	ND	ND	ND	79.30
ΔH **[J/g]**	ND	ND	ND	8.00
T_g **[°C]**	37.40	36.60	36.10	26.70
M_n **[kDa]**	43.90	10.20	8.50	1.70
D	1.93	3.13	2.67	3.96
WU **[%]**	0.00	2.50	3.50	45.20
WL **[%]**	0.00	1.10	1.10	50.30

EB-P(L-LA:GA:TMC) rod-ARP

Time [Days]	0	28	42	84
F_{LL} **[mol%]**	59.40	59.60	49.40	47.00
F_{GG} **[mol%]**	17.50	16.60	16.10	20.40
F_{TMC} **[mol%]**	23.10	23.80	34.50	32.60
T_m **[°C]**	ND	ND	ND	79.60
ΔH **[J/g]**	ND	ND	ND	9.40
T_g **[°C]**	35.60	36.40	33.70	29.40
M_n **[kDa]**	43.40	8.50	3.80	1.50
D	2.01	2.40	2.56	1.86
WU **[%]**	0.00	6.10	9.20	59.70
WL **[%]**	0.00	0.80	1.30	65.50

Conclusions

The way of ARP release indicates that the proposed formulations may be administered as a delayed-release system. EB irradiation was found to accelerate ARP release.

Acknowledgments

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GROWTH AND OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE-DERIVED STEM CELLS ON $Ti_3C_2T_x$ MXenes, NEWLY EMERGING 2D NANOMATERIALS FOR BIOMEDICAL APPLICATIONS

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Introduction

MXenes are one of the groups of newly discovered nanomaterials with a 2D structure. They are transition metal carbides/nitrides with the general formula $M_{n+1}X_nT_x$, where M represents the transition metal (e.g. Ti, Mo, or Cr), X represents carbon and/or nitrogen and T_x denotes the surface termination (e.g. -F, -OH or =O). Due to their interesting electrical, mechanical, and optical properties (e.g. thermal and mechanical stability, high electrical conductivity, easy surface functionalization, strong fluorescence efficiency), MXenes are perspective nanomaterials in a variety of applications, e.g. catalysts for hydrogen production, adsorbents of heavy metals from the environment or electrochemical and optical sensors [1]. This new nanomaterial is also suitable for medical applications as a biosensor detecting neural cell activity, cancer biomarkers, or molecules of DNA [2]. MXenes have been shown to stimulate osteogenic differentiation [3,4].

In our experiments, we compared three types of MXene layers with various surface modifications (i.e. pristine, -COOH, and $-NH_2$ terminal groups) on tissue culture polystyrene in terms of their ability to promote adhesion, proliferation, and osteogenic differentiation of human adipose tissue-derived stem cells (ADSCs).

Materials and Methods

$Ti_3C_2T_x$ MXene was synthesized by selective etching of aluminum atomic layers in MAX phase Ti_3AlC_2, as described elsewhere [5]. MXene layers were characterized by UV-Vis and Raman spectroscopy and also with TEM analysis.

Human ADSCs were obtained from a healthy woman donor by liposuction. The cells were expanded in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 10 ng/mL of fibroblast growth factor2 (FGF-2).

MXenes were sterilized in UV light. Materials were seeded with ADSCs in DMEM containing 10% of FBS and 10 ng/mL of FGF-2. The cells were cultivated on samples for 7 days. Cell counting was done on days 1, 3, and 7. Osteogenic differentiation potential was examined by ADSCs cultivation on three types of MXenes in DMEM containing 10% FS, b-glycerolphosphate, ascorbic acid, dexamethasone, and vitamin D3.

The samples were fixed in ice-cold 70% ethanol. Filamentous actin in cells was visualized by phalloidin-TRITC, and cell nuclei were counterstained with DAPI. ADSCs grown in an osteogenic differentiation medium were immunostained using anti-collagen I goat polyclonal Ab (ab19811, Abcam; visualized with Alexa 488). Cell nuclei were counterstained with Hoechst 33258. Fluorescent images were taken on Olympus epifluorescence microscope IX71 (DP71 digital camera, objective magnification 10×).

Markers of osteogenic differentiation were examined by the qPCR method and their expressions related to that of beta-actin were determined.

Results and Discussion

The ADSCs adhered well to all types of MXene modifications, showing the best adhesion and proliferation potential on MXene with -COOH surface modification (FIG. 1). This may be related to the electrostatic interaction between negatively charged -COOH groups of MXenes and positively charged phosphatidylcholine lipids in the cell membrane. On the other hand, NH_2 modification was unsuitable for cell growth - the number of cells increased only slightly between days 1, 3, and 7.

The expression of osteogenic markers, namely the genes for type I collagen, alkaline phosphatase, and Runt-related transcription factor 2 (COL1A1, ALPL, and RUNX2, respectively) was increased on MXenes, independently of the surface modification. This suggests that the osteogenic potential of MXenes is caused by their mechanical properties rather than their surface termination groups.

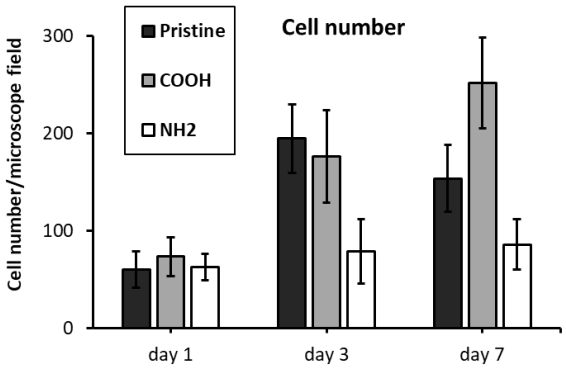

FIG. 1. Number of ADSCs on MXene layers.

Conclusions

The pristine and -COOH MXene layers have been shown to support cell adhesion and proliferation, whereas $-NH_2$ MXene supported the proliferation only slightly. MXene layers can promote osteogenic differentiation independently of their surface termination groups.

Acknowledgments

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NEW POLYMER MATERIALS BASED ON CITRIC ACID SHOW PROMISING PROPERTIES FOR MANUFACTURING SMALL DIAMETER VASCULAR GRAFTS

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Introduction

Cardiovascular diseases represent a major problem for the healthcare systems of most developed countries and remain their leading cause of mortality. Even though vascular grafts have been created from synthetic polymers already since the 1950s [1], and these materials have undergone a long way of optimizing their chemical and mechanical properties, biocompatibility, etc., still some limitations persist in case of small diameter vascular grafts (< 6 mm), which are prone to restenosis. This problem can be solved by constructing of tissue-engineered vascular grafts based on autologous patient´s cells. For this purpose, we have developed a material based on poly(hexamethylene citrate) with glutathione, which exhibits properties favourable for the adhesion and proliferation of human adipose tissue-derived stem cells (hADSCs).

Materials and Methods

Poly(hexamethylene citrate) (PHC) materials were obtained in two steps as previously described [2]. The biocompatibility of the material was then tested with hADSCs obtained from high-pressure liposuction of a healthy patient in maximal passage 3. For the cultivation of cells, we used Dulbecco's Modified Eagle's Medium (DMEM) with the addition of fibroblast growth factor 2 (FGF2) and a mixture of antibiotics/antimycotics.
Since cardiovascular diseases are often associated with increased oxidative stress, PHC was also enriched with glutathione (GSH), known for its antioxidant properties, in the concentration range from 0 to 1.6 wt.% (modification of PHC materials with GSH was conducted at the postpolymerisation step). The cells on the materials were then treated with an oxidative stress inducer menadione [3] in a concentration- and time-dependent manner, and the number and/or metabolic activity of the survived cells were measured.

Results and Discussion

Before seeding the cells, PHC was thoroughly washed with the culture medium in order to decrease its acidity. However, the acidity of the media decreased with the number of washout repetitions. Another advantage of our materials was their transparency, which enables real-time monitoring of living cells. Nevertheless, this transparency slightly decreased with GSH concentration.
hADSCs seeded on tested materials with/without GSH showed relatively high adhesion and proliferation. We assume that the materials were able to capture FGF2 from washouts prior to cell seeding and then release it back into the media during cell growth. This is an advantage over currently used synthetic polymers, often bioinert and unsuitable for cell growth. The positive influence of GSH addition on oxidative stress was proved using the resazurin metabolic test – the cells seeded on tissue culture polystyrene (TCPS) showed a deeper relative decrease in metabolic activity (FIG. 1).

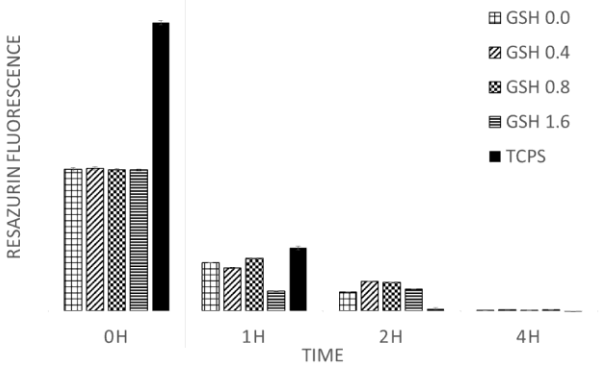

FIG. 1. Metabolic activity (resazurin fluorescence) of hADSC seeded on PHC (with --/0.4/0.8/1.6 wt. % GSH) and TCPS respectively after menadione (500 µM) treatment.

A comparison of the cell proliferation rate on TCPS and PHC (-/GSH) still comes out more favorable for TCPS (FIG. 2). However, our material shows more suitable mechanical properties for the production of tissue grafts. Considering our plans to differentiate hADSCs into smooth muscle cells, moderate proliferation is advantageous due to a lower risk of graft restenosis.

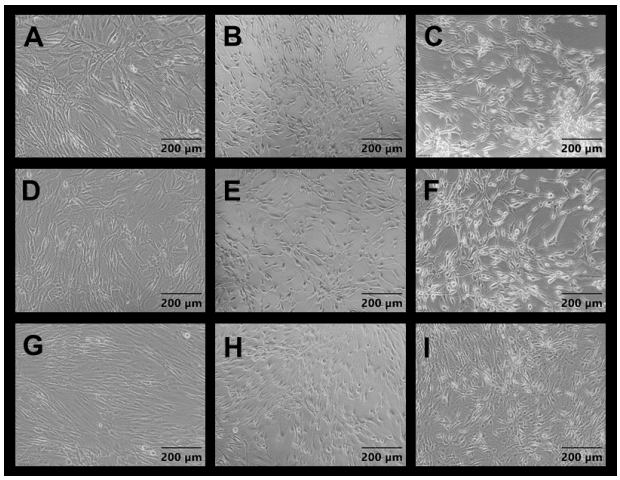

FIG. 2. A, B, C – cells on PHC(-) material after 0h, 1h, 2h respectively menadione (500 µM) treatment; D, E, F – cells on PHC (0.8 µM GSH) in the same time points of menadione treatment; G, H, I – cells on TCPS after the same menadione treatment.

Conclusions

Our developed PHC materials with/without GSH show properties suitable for the formation of small diameter vascular grafts as elasticity, transparency and biocompatibility with moderate cell proliferation, which reduces the risk of restenosis.

Acknowledgments

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THE EFFECT OF SLM SURFACE MODIFICATION OF Co-Cr-Mo ALLOY IN MATERIAL AND THERMAL ENGINEERING

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Introduction

The subject of thermal energy transfer and cooling systems is still a matter of contention in many different fields of science and industry. Yet, thermal conductivity is still not a popular research parameter in engineering of biomaterials. Using laser to modify metal's surface is supposed to increase the heat change capacity and efficiency and, therefore, increase the metal's adjustment to the body temperature in order to minimize the feeling of a strange cold object in the body system. Different modification patterns made with the usage of laser are expected to show a repeatable trend in thermal conductivity. Effects are going to be measured, among others, by the analysis of changes in Reynold's and Nusselt's numbers, similar to those described by Burgess and Ligrani [1] or Isaev et al. [2]. Results are expected to show the best pattern to increase the heat conduction in Co-Cr-Mo alloys [3]. They are not very many examples on this particular subject matter. The initial phase of laser modifications has been conducted using trial and error method based on the general material properties knowledge. Thermal properties of Co-Cr-Mo alloys are still not very well-examined, since most researchers focus on its biocompatibility and durability and conduct mostly structure and durability tests.

Materials and Methods

In order to perform the SLM surface modification of Co-Cr-Mo alloy base for cladding, an industrial 3D printer ReaLizer 100, which melts and fuses metallic Chrome-Cobalt powder with a laser beam was chosen as the Selective Laser Melting (SLM) solution for an effective surface modification. Using semi-material process (similar material for both cladding base and SLM-printable powder) helped to avoid formation of a galvanic cell between the base surface and the cladded structure. The size of the spherical Chrome-Cobalt powder particle was chosen as up to 50 µm. The best process parameters for the Co-Cr powder were found and described by T. Seramak et al., as: laser power in range of 45-75 W, spot size of the laser beam 0.13 mm, layer thickness 25 µm. The working chamber was filled with argon as a protective atmosphere, the level of oxygen was reduced to 0.2% [3]. Five different patterns were designed in the Autodesk AutoCAD program and then converted to the *.stl format compatible with the device.
Constant conduction of interdisciplinary consultations creates the need of implementing simple computer tools, which support information exchange and general collaboration [4]. Moodle is an LMS (Learning Management System) tool, which in this case is also used as a discussion platform and a results' repository. All the information stored on Moodle is password protected and made available only to a limited group of researchers involved in the project.

Results and Discussion

Samples were pre-examined both on an optical microscope and JEOL scanning electron microscope (SEM) a with an EDX X-ray generator. No contraindications or concerns considered with their arrangements for further examinations have been discovered. Diffractogram results confirmed that the main elements contained in the powder were chromium and cobalt. A high content of wolfram was also detected. Next, they were sent to the Institute of Energy of Gdańsk University of Technology for the heat coefficient and fluid transfer parameters' tests on an independent self-designed measuring apparatus. The device, which is going to be used, is an original project, designed and build by scientists of the Gdansk University of Technology. The project of the measuring device was described by the authors, Muszyński T., Andrzejczyk R. [5-6].

Conclusions

Different surface modification patterns applied with the use of pulse and SLM lasers are expected to show a repeatable trend guiding to the future conclusions, narrowing the scope of research and optimization of the further phases of the experimental study.
The effects are going to be measured by comparing the surface roughness, inner structural changes, heat and liquid flow parameters, chosen based on literature [7-8], such as heat transfer coefficients, Nussel and Reynolds numbers etc. of the modified samples to the smooth reference sample of each material. Discovering the most effective pattern for the best possible improvement of the heat transfer coefficient will facilitate the cooling devices' size reduction and their infrigidation effectiveness.

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SILK FIBROIN MODIFIED CORE-SHELL FIBERS FOR CORNEA REGENERATION

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Introduction

The possibility of modifying the surface properties and topography of the original fibers are the main advantages of the coaxial electrospinning. Due to specific morphology, the core-shell fibers could play a universal role of the scaffold for the regenerated tissue and the carrier for the drugs, proteins, growth factors etc. Additionally, the faster degradation of one of the core-shell fiber layers, in addition to releasing the embedded additive, also leads to a uniform reduction in fiber diameter and an increase in porosity. As a result, better light transmission and cells migration into the scaffold can be obtained. In turn, the introduction of proteins into one of the layers improves biocompatibility and reduces the pro-inflammatory effects of the scaffold [1-3].
In this study, the native PCL fibers were obtained by the coaxial electrospinning, the shell was modified by silk fibroin to enhance the biological effect of the scaffold. In the first part of the work, electrospinning conditions were defined to obtain a nanotextured surface. In the second part of the experiment, the effect of core-shell fiber morphology enriched with fibroin, on the optical properties and biological performance of the scaffold was investigated. The potential of core-shell fibers as carriers in bioactive molecule delivery systems was confirmed. In addition, the effect of silk fibroin on cell viability was also evaluated.

Materials and Methods

As a core part, 10% (w/v) Polycaprolactone (PCL, Mw = 80kDa, Sigma-Aldrich) dissolved in a solution of dichloromethane (DCM, Chemland SA) and dimethylformamide (DMF, Chemland SA), was used. Part of the shell was 8% Polyvinylpyrrolidone (PVP, Mw = 1300000, Acros Organics) dissolved in a solution of ethanol (EtOH, Chemland SA) and DMF. The microstructure of nanofibers were observed with a scanning electron microscope (NOVA NANO SEM 200). To determine the physicochemical properties of a scaffold, contact angle and surface free energy was measured by goniometer (DSA 25 Kruss). The presence of the silk fibroin was checked by ATR mode in FTIR study (Bruker Tensor 27 FTIR). The release kinetics of protein in the material was assessed by spectrophotometry (UV–Vis, Shimadzu 1900i). The light transmittance of the nonwoven fabric was evaluated by spectrophotometry (UV-Vis equipped with a sphere, Jasco V-630). Cytocompatibility was determined by direct contact of the fibrous scaffold with fibroblasts and assessed after 3rd and 7th day of the in vitro incubation.

Results and Discussion

The SEM observations showed the presence of the core-shell fibers with morphology referred in the literature as *beads-on-string* (FIG. 1). Such a microstructure enabled to obtain a fibers core with diameter in a range of 80-300 nm (average 0.14 µm ± 0.03) for both samples consisting fibroin and references (without fibroin). As for the shell part, the samples consisting fibroin was larger (average 1.98 µm ± 0.5) than the reference sample (average 0.88 µm ± 0.15). The small diameter of the fibers was reflected in the light transmission studies. All of the samples exhibited high light transmission in a wet state (>80%). In the dry state, the transmission was ~60% for the 10µm thick samples. Due to the presence of the PVP in the shell part, the contact angle of the samples decreased from 130° characteristic for native PCL fibers to ~50° for PCL/PVP samples and ~40° for silk fibroin consisting samples. It was also reflected in the release kinetics studies. Most of the silk fibroin was released from the substrate in the first 24 h due to the burst release effect. All of the samples exhibited high viability of Hs 680 fibroblasts. None of the tested scaffolds showed a cytotoxic effect.

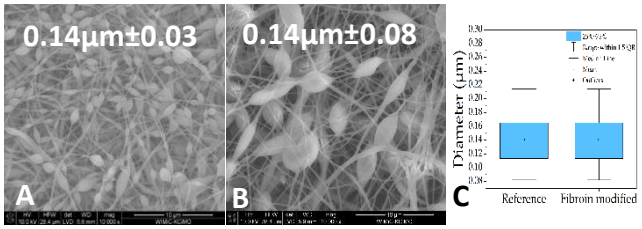

FIG. 3. Microstructure of the reference (A) and fibroin (B) modified samples. Box chart presenting the fibers size (C).

Conclusions

The coaxial electrospinning process makes it possible to obtain fibers with modified nanotopography and bioactive molecules. The presence of PVP with silk fibroin in the coating enabled obtaining small fibers with a narrow size distribution, which contributed to good optical transparency of the scaffold, which is essential for corneal implants. In addition, rapid degradation of the outer layer (shell) leads to increased porosity and rapid release of silk fibroin, which in turn contributes to better cell viability on the silk fibroin-modified scaffold.

Acknowledgments

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DIFFERENTIAL ANTIBACTERIAL ACTIVITY OF POLYSACCHARIDE BASED WOUND DRESSING

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Introduction

Currently on the market there are about 6k MD products for wound management [1-4]. The effectiveness of treatment as well as the comfort and health of the patient depend on the choice of the appropriate wound dressing. Most health organizations, that prepare national recommendations and guidelines for wound care, agree that there is no single agreed set of criteria for assessing the effectiveness and quality of wound dressings. However, the features that an ideal dressing should meet are well established [1-7].

Electrospinning is one of the most promising method for obtaining new generation, active materials - an ideal component of a controlled release system for drugs, biologically active ingredients, or other substances that have a therapeutic effect or support the regeneration of diseased or damaged tissue. The nanofibers are characterized by a relatively large total specific surface area, ranging from 10 - 10^3 m^2/g for fibers with a diameter of about 500 nm [8] which enables incomparably larger contact surface with the external environment than in the case of fibers or traditional materials.

The objective of the study was to prepare an active wound dressing materials that would reveal differential antibacterial activity, designed for chronic wounds with different etiology. In our research polysaccharide based (sodium alginate) wound dressing materials with differential antibacterial activity were obtained. As biologically active agents silver nitrate, zinc oxide and flake graphene oxide were used.

Materials and Methods

In the studies alginic acid sodium salt from brown algae (Sigma – Aldrich) with viscosity 5.0 - 40.0 cps for c = 1% in water 25°C was used as base polymer. Obtained materials were crosslinked and modified in order to receive differential antibacterial character. As modifiers, for bulk wound dressing modification as well as for polymer crosslinking, such agents as: silver nitrate extra pure (Sigma - Aldrich), zinc oxide powder (Idalia), calcium chloride (Idalia) and graphene oxide flakes in dispersion (Łukasiewicz Research Network - Institute of Microelectronics and Photonics – FIG. 1) were used.

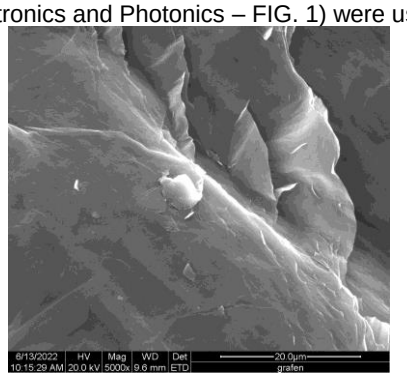

FIG. 1. SEM image of graphene oxide flakes.

Prototype wound dressing materials, ultrafine fibres based, were prepared by electrospinning an aqueous sodium alginate solutions using robotic electrospinning machine TL-Pro-BM (TONG LI TECH -PRC).

Materials were characterized using techniques such as scanning electron microscope (SEM) for characterization of the macroscopic structure and surface, the infrared spectrometer (FTIR) for spectral characteristics of each samples. In order to determine active character antibacterial test were performed in Microbiological Laboratory (Lukasiewicz Research Network - Lodz Institute of Technology).

Results and Discussion

Result of preliminary studies indicate that both proposed methods of antibacterial functionalization are promising. Either adding active ingredients into a polymer matrix (bulk modification) like zinc oxide, graphene oxide flakes (FIG. 2) or crosslinking sodium alginate dressing by silver nitrate, calcium chloride affect obtaining materials with different antibacterial activity.

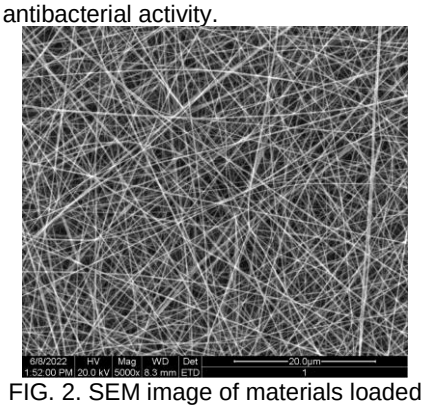

FIG. 2. SEM image of materials loaded with graphene oxide flakes.

Conclusions

The preliminary trials succeeded in obtaining the optimal composition and concentration of the spinning liquid which allows its processing through electrospinning. Simultaneously, dressing materials with differential antibacterial activity were obtained.

Acknowledgments

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SELECTION OF PARAMETERS FOR THE PRODUCTION OF MAGNESIUM POTASSIUM PHOSHPATE CEMENT WITH APPROPRIATE PROPERTIES FOR MEDICAL APPLICATIONS

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Introduction

Bone cements are a group of biomaterials that are widely used in orthopaedic surgery and traumatology. These materials are created by mixing powder and liquid, which results in a mouldable mass that sets when applied to its destination. They are also characterized by an important advantage - injectability, which significantly extends their use in minimally invasive surgical procedures. Currently, the most commonly used types of cements are ceramic or polymer, but both groups have deteriorating disadvantages [1,2]. Acrylic cements (based on PMMA) release unreacted toxic MMA monomer and have a high temperature of polymerization. Moreover, they are non-degradable. On the other hand, ceramic cements (the most commonly used nowadays are calcium phosphates) have long setting time, low mechanical properties and poor injectability [3]. The results of studies from recent years show that cements based on magnesium phosphate are characterized by initial high mechanical properties, faster setting time, more effective resorption time and good biocompatibility compared to calcium phosphates that are used today [4]. The aim of this research was to analyse the influence of selected parameters of bone cement production, i.e. the ratio of magnesium to phosphorus (Mg/P ratio) and the ratio of powder to liquid (P/L ratio) on its main properties, such as: setting time, microstructure, phase composition, microhardness, setting temperature, surface wettability, injectability, degradation rate and cytocompatibility.

Materials and Methods

The cement powder used for the study was consisted of dead-burned magnesium oxide (AmBeed, USA; 1500°C, 5h; particle size ~52,75 μm) and potassium phosphate (Chempur, Poland; particle size ~78,08 μm) mixed in various molar ratios (Mg-P - 3-5 : 1), while as a liquid a demineralized water was applied (P-L – 2-3 : 1). After adding the powder to the liquid, the resulting paste was mixed until a homogenous paste was obtained, usually for 45 seconds. The setting time of the cement was assessed using the Vicat apparatus, while the setting temperature was measured with a thermocouple. Microhardness was examined with a Vickers microhardness tester. The microstructure of the material was assessed using scanning electron microscope, phase composition was evaluated by X-ray diffractometer and surface wettability was tested on optical tensiometer. The degradation rate was checked after a biweekly and monthly incubation in the PBS solution (phosphate-buffered saline, 37°C). The injectability of the studied cements was qualitatively determined by manual squeezing the pass out of the surgical syringe and then assessing the level of injection of the pass before it was fully set. The *in vitro* cytocompatibility study was performed on human osteoblast cell line (hFOB 1.19, ATCC) using the standard MTT assay. The following Table shows examined Mg/P and P/L ratios:

-	P/L ratio			Mg/P ratio		
Mg/P ratio	4:1			3:1	4:1	5:1
P/L ratio	2:1	2.5:1	3:1	2.5:1		

Results and Discussion

The result of the research was the selection of favourable parameters for the production of cement based on magnesium potassium phosphate - the setting time which is less than 25 minutes and the setting temperature below 50°C - which is extremely important for application reasons in clinical practice [5]. In the surface wettability tests, contact angles lower than 15º were obtained, which indicates a significant hygroscopicity of the studied materials. The change of Mg/P and P/L ratios have significant influence on cement reaction as the increase of those ratios caused a decrease of setting time and increase of setting temperature. There was no direct correlation between the microhardness of the measured specimens and their above ratios, possibly due to the variable and random porosity of cements. The degradation results showed that with an increase in the Mg/P and P/L ratio cause the reduction of mass loss, hence improved the biostability of cements. The specimens with Mg/P ratio 3:1 and P/L ratio 2.5:1 were characterized by the best injectability. Examination of the microstructure shows the different areas consisted of agglomerations of cement crystals, smooth area (possibly of amorphous phase), pores and also cracks (probably due to drying the specimens). Two phases were observed in the XRD diffractometers: $KMgPO_4$ x 6 H_2O and unreacted MgO. The biological study demonstrated that the 3:1 for P/L ratio and 4:1 for Mg/P ratio have the most favourable cellular response.

Conclusions

The most optimal properties of cement were obtained for a 2.5:1 powder to liquid ratio (with Mg/P ratio of 4:1) and for a Mg to P ratio of 4:1 (with powder to liquid ratio of 2.5:1), cause of the most optimal parameters of the production of magnesium phosphate cement.

Acknowledgments

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ROLE OF ADIPOCYTES IN HEALING OF THERMAL SKIN DAMAGE WITH UNDERLYING SOFT TISSUES

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Introduction

Thermal injury of the skin with underlying soft tissues is common in both civilian and combat scenarios [1]. Healing patterns of skin wound are determined by participation of various cell types. Adipose tissue is an organ that performs a lot of significant physiological functions [2]. The role of adipose tissue in skin wound healing is a debatable issue that requires comprehensive research. The aim of the study is to reveal the role of adipocytes in the healing of experimental low-temperature and high-temperature lesions of the skin with underlying soft tissues.

Materials and Methods

In this study, the authors carried out an experiment on 30 rats of WAG line. Two groups were formed. Group 1 included 15 rats, in which a cold wound was modeled on the lateral surface of the thigh. Group 2 included 15 rats, which had a thermal burn modeled on the lateral surface of the thigh. Animals were withdrawn from the experiment on the 14th and 21st day.

The material of the study was the skin with underlying soft tissues. The slides were stained with hematoxylin and eosin. An immunohistochemical study was performed using mouse monoclonal antibody (MCA) to α-smooth muscle actin (clone 1A4, DAKO, Denmark) (α-SMA). The slides were studied on an Olympus BX-41 microscope (Japan), followed by processing with the Olympus DP-soft version 3.1 software package. A morphometric study was carried out with it. Absolute number of adipocytes, as well as the relative number of adipocytes which expressed α-SMA (%) were determined in microscope field of view ×100. Mann-Whitney U-test was used for statistical analysis.

Results and Discussion

Survey microscopy of the slides in groups 1 and 2 revealed a wound cavity filled with granulation tissue on days 14 and 21 of the experiment. Single adipocytes or groups of adipocytes were determined in granulation tissue. The absolute number of adipocytes increased ($p<0.05$) in both groups on the 21st day compared to the 14th day (TABLE 1). Adipocytes were often localized in granulation tissue near the proliferating epidermis that covered the wound surface. Interestingly, in the granulation tissue around the accumulation of adipocytes, many vessels were noted, which, accordingly, improved trophism and contributed to the rapid maturation of granulation tissue. The identified microscopic findings indicate that adipocytes may stimulate proliferative processes in the epidermis and activate the vasculogenesis.

In groups 1 and 2 on day 14 the adipocytes were of round, oval, oblong or sometimes fibroblast-like shape. On day 21 in both groups the number of adipocytes with oblong or fibroblast-like shape increased.

An immunohistochemical reaction revealed adipocytes that expressed MCA to α-SMA in both groups. This MCA was predominantly expressed by adipocytes of oblong or fibroblast-like shape. On day 21, as compared to day 14, the relative number of adipocytes expressing MCA to α-SMA increased ($p<0.05$) (TABLE 1).

TABLE 1. Results of the morphometric study.

Indicator name	Day of the experiment	Group 1	Group 2
Absolute number of adipocytes	14	53.9±2.4	34.2±1.6 *
	28	74.4±3.9 #	51.8±2.1 *#
Relative number of adipocytes, which expressed α-SMA, %	14	19.3±1.1	10.4±1.8 *
	28	32.2±1.3 #	21.6±2.3 *#

* - significant differences compared to group 1;
\# - significant differences compared to the indicator of the previous experimental term.

The changes in adipocytes shape and their expression of α-SMA indicated that these cells can turn into myofibroblasts. The latter are known to produce connective tissue fibers and promote the wound contraction [3]. Qualitative and quantitative changes of adipocytes in both groups occurred against the background of granulation tissue maturation and its transformation into connective tissue.

In group 2, compared with group 1, we found lower ($p<0.05$) values of the absolute number of adipocytes in the granulation tissue and the relative number of adipocytes expressing α-SMA.

Conclusions

The study showed the role of adipocytes in the healing of skin wounds caused by temperature exposure. Adipocytes activate proliferative processes in the epithelial layer, induce vasculogenesis and have the ability to turn into myofibroblasts. A comparative analysis has shown that adipocytes are of greater importance in the healing of low-temperature lesion of the skin with underlying soft tissues and of lesser importance in the healing of high-temperature lesion of the skin.

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MODIFICATION OF MAGNESIUM PHOSPHATE BONE CEMENT WITH 2-HYDROXYETHYL METHA-CRYLATE

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Introduction

For centuries, the idea of supporting bone tissue repair has emerged, but still, bone healing is a considerable challenge in today`s clinical routine. A variety of synthetic bone substitutes have been developed, but a particularly interesting group that deserves attention are bone cements. Generally, these biomaterials have two components – powders and liquid, and after mixing them, a workable and injectable paste with hardening properties is obtained. Two groups of bone cements are mainly used: ceramic (based on calcium phosphates) and acrylic (mainly based on poly(methylmethacrylate)) [1]. More recently, it has been seen that a promising alternative to traditional ceramic cements may be materials based on magnesium phosphate (MPC). Comparing with calcium phosphate, they set quickly, have high initial strength, resorb much faster, and demonstrate greater osteogenic potential. However, the MPC cements, like every biomaterial, has its drawbacks, like: high temperature setting reaction, difficult injectability and susceptibility of the paste to leaching [2,3]. Therefore, the novel cement formula was proposed here and is based on MPC modified with 2-hydroxyethyl methacrylate (HEMA).

Materials and Methods

Tri-magnesium phosphate powder was obtained by sintering mixture of $MgHPO_4 \cdot 3H_2O$ and $Mg(OH)_2$ in 2:1 molar ratio at 1100°C for 5 h, then crushed, ground and sieved. Next, this powder was mixed in 4:1 mass ratio with finely ground 0.5 M di-ammonium hydrogen phosphate. The cement liquids were water solutions of HEMA including 2.5 µl/mL tetramethylethylenediamine (TEMED). 2.5 µg/mL ammonium persulfate (APS) was added to solutions to start the hydrogel cross-linking reaction. The used powder-to-liquid ratio was 2.5 g/mL. The cement specimens were prepared by premixing HEMA solutions with APS activator for different premix times (2:30 or 4:00 min) and then added to cement powder and manually stirring until obtained a homogeneous paste. Next, the paste were transferred into silicone rubber molds and stored for 24 h at 37°C and > 90% humidity (water bath). As reference, the cement powder was mixed with water and treated identically to the tested cements. The following properties were evaluated: setting time, microstructure (SEM microscopy), phase composition (XRD diffractometry), porosity, compressive strength, and degradation behaviour (immersion in the PBS solution for 18 days). Moreover, the cytocompatibility of modified cements was evaluated with human osteoblast cell line (hFOB 1.19, ATCC) colorimetrically by the MTT assay after three days of culture.

Results and Discussion

In this research, a novel dual-setting cement based on MPC and HEMA was developed. The addition of the hydrogel component significantly influenced the main properties of the magnesium cement by shortening its setting time, reducing its initial porosity, improving its mechanical properties, but also worsening the cellular response. As a result of the initial screening tests, three concentrations of HEMA content were selected, which allow to obtain cement with optimal properties: 15, 20 and 25%. For the modified cement, the setting time was between: 16-21 min (control ~22:33), initial porosity: 2.6-4.2% (control ~5%), final porosity: 6.5-11.5% (control ~7.2%), mass loss: 0.1-3.5% (control ~0.3%), compressive strength: 39.8-64.1 MPa (control ~50.1 MPa), Young Modulus: 2,1-3,2 GPa (control ~ 2,3 GPa) and cell viability: 22-54% (control 100%). In the SEM analysis, it was found that both HEMA content and premix time significantly influenced the formation of hydrogel agglomerates in the cement matix, which results in differences in the above properties. Moreover, shorter premix time contributes significantly to mass loss after degradation in the PBS solution compared to the control and longer premix time. Based on XRD analysis, no phase changes after the modification of cement were found - cements were consisted of struvite, farringtonite, newberyite and unreacted ammonium hydrogen phosphate. A more effective improvement in the mechanical properties of modified cements was found for a longer premix time, however for the highest content of HEMA (in longer premix time), the effect was opposite. The negative influence of the HEMA on the cellular response of MPC cement was related to polymer itself, which was found in the additional cytotoxic study of the hydrogel material and it is assumed that probably was caused by the used cross-linking agent (TEMED). Hence, despite obtaining favorable physical and mechanical properties of the modified cement, future research should focus on the selection of a different hydrogel additive or HEMA cross-linking process.

Conclusions

Based on results of this research, it is assumed that the longer premix time (4:00 min) and the medium HEMA concentration (20%) show the most favorable effect on the cement properties and the applied modification may allow to obtain a novel dual-setting cement with better physical and mechanical properties.

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CHARACTERIZATION OF 3D STRUCTURES BASED ON CARBOXYMETHYL CHITOSAN

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Introduction

Due to the zero-waste trend, polymers made from post-production waste have recently become popular. One of the representatives is carboxymethyl chitosan (CMCS) with unique properties such as biodegradability, biocompatibility, no cytotoxicity, and antibacterial properties [1-3]. In addition, CMCS is a hydrophilic derivative of chitosan and a significant amphoteric [4-6]. Thus, this polysaccharide can be considered as a good candidate for the fabrication of new functional and structural materials. To be used in tissue engineering, it must be mixed with a water-insoluble polymer or the possibility of crosslinking should be used. There are three options for crosslinking polymers: using a crosslinking agent - chemical crosslinking, using UV irradiation with a specific beam - physical crosslinking, and enzymatic crosslinking. This study was aimed to investigate the influence of two cross-linking agents on the surface properties of 3D carboxymethyl chitosan structures.

Materials and Methods

Materials

Low molecular weight carboxymethyl chitosan (CMCS) was purchased from Heppe Medical Chitosan GmbH (Halle, Germany). Tannin acid (TA), was received from Pol-Aura (Dywity, Poland) and citric acid (CA) was bought from Chempur (Piekary Śląskie, Poland). All chemicals were used as received without further purification.

Methods

Carboxymethyl chitosan was dissolved in water, then some poured into a 24-well plate and frozen. In the next step, the appropriate amount of tannic acid solution (1%) was added to the carboxymethyl chitosan solution, then the obtained solution was homogenized for 3 minutes. In the next step the CMCS/TA solution was poured into a 24-well plate. Similarly, 3D structures were made with the use of citric acid (1%). The samples were then frozen at -20°C and freeze-dried.

The following analyses were performed:

- TR-FTIR analysis, VERTEX 70v FT-IR Spectrometer (Brucker Optics Inc), in the wavelength range between 4000 – 400 cm^{-1}, resolution of 2 cm^{-1} and 60 – times scanning.
-SEM analysis, Quanta 3D FEG, D9399, FEI Company, Eindhoven, the Netherlands.
-TGA analysis, Jupiter STA 449 F5 thermoanalyzer by Netzsch with an automatic sample feeder coupled with the FT-IR Vertex 70V spectrometer by Bruker Optik.

Results and Discussion

ATR-FTIR analysis showed a significant reduction of the bands for CMCS/CA relative to the 3D structure of chitosan. A smaller difference in surface areas was observed for CMCS/TA. However, the DTG curves of obtained scaffolds showed no significant difference between CMCS/CA and CMCS scaffolds. In the case of CMCS/TA scaffold, the joining of two peaks indicating polymer degradation was noted. This proves a good crosslinking of the CMCS/TA scaffold.

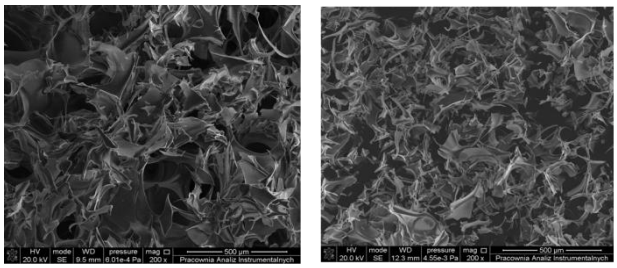

FIG. 1. Representative SEM images of 3D structures.

Morphology of obtained scaffolds were analysed by using scanning electron microscopy (SEM). Images were made under 200 magnification (FIG. 1). For CMCS/TA scaffold, it was found that the sample homogeneity increased, and the number of large pores in the 3D structures decreased. The structure of the CMCS/CA was less homogeneous than that of the CMCS/TA.

Conclusions

Comparing the effect of two crosslinking agents: citric acid and tannic acid on the 3D structure of carboxymethyl chitosan. It can be concluded that the CMCS/TA scaffold was characterized by better properties.

Acknowledgments

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SONOCHEMICAL FUNCTIONALIZATION OF POLYMERIC MATERIALS WITH NANOPARTICLES OF BIOACTIVE MOLECULES

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Introduction

Controlled site-specific drug delivery from the implantable materials offers an attractive and long-awaited alternative to oral, parenteral, and topical modes of administration. site-specific, controlled drug delivery is especially desirable where clinicians wish to elicit a localized therapeutic pharmaceutical effect. Such delivery allows achieving the therapeutic dose at the desired site of action while maintaining a low or negligible systemic level of a drug. Although there are numerous reports on the functionalization of polymeric surfaces with drugs in the context of their usage as drug delivery systems, the sonochemical embedment of bioactive substance nanoparticles is underestimated and still unexplored. This technology enables the insertion of active substances directly into the surface layer of the material and is an attractive alternative to therapeutic coatings releasing drugs based on the phenomenon of degradation of the polymer matrix. The adjustment of the ultrasound radiation parameters (time, power, pulse, amplitude) provides the possibility of tuning the size of generated nanoparticles and their embedment depth [1].
Due to the extraordinary speed and complexity of this process, the exact mechanism of the formation of nanoparticles under the influence of ultrasound is still discussed. Molecular dynamics (MD) is a widely used method to study the interfacial phenomena at the molecular level, often employed in parallel with experimental methods. With recent advances in computational power and simulation methodology, MD is particularly successful in studying biologically relevant interfacial processes. It is a well-suited method for exploring the details of nanoparticle formation in the ultrasound treatment and providing support and feedback for our experimental methods.
Such a strategy, combining the precise molecular-level insight with state-of-the-art micro-and macroscopic experimental approaches, is intended to improve surface biocompatibility and targeted drug delivery applications.

Materials and Methods

Molecular dynamics (*MD)* simulations were performed using fine-tuned AMBER fully-atomistic force field [2]. TIP3P water parameters were used [3], and the OPC4 water model, reproducing experimental water surface tension. The force field parameters for fluorouracil (model drug molecule) molecules were developed. MD simulations were performed using the GROMACS simulation package [4]. The simulation system contains the interface between a nanometric bubble (R = 2 nm) and fluorouracil, ethanol and/or water molecules. The simulation length for all simulations was 100 ns. For analysis of trajectories, standard GROMACS tools were employed
Sonochemical synthesis parameters of nanoparticles of bioactive substances were optimized. *TEM* observations were performed to characterize nanoparticles size and morphology (TITAN 80-300, FEI). The stability of bioactive compounds under sonochemical synthesis was tested using *ATR-IR* spectroscopy (Nicolet 6700).

Results and Discussion

The systems for MD simulations were built to follow the experimental systems. Typically different basic solutions were studied with different ethanol/water ratio.
To compare the effect of the bubble interface inside the simulation box, 4 different situations were considered: water/ethanol solutions, water/ethanol solutions with bubble, model drug molecule/water/ethanol solutions, model drug molecule/water/ethanol solutions with bubble.
For low concentration of ethanol in water we observed that ethanol molecules are preferentially accumulating on the bubble surface providing suitable interface for drug agglomeration.
The highest effect was observed for the smallest concentration of ethanol in water. This indicates the optimal conditions for water soluble drug nanoparticles formation. The next step was to simulate the bubble collapse which takes place during the sonication of the solution. The aggregation of drug molecules after bubble disintegration was observed and was considered an early stage of NPs nucleation. In parallel, experimental studies the sonochemical experiments were designed based on the MD simulation and the TEM images of nanoparticles formed from drug molecules are presented in FIG. 1.

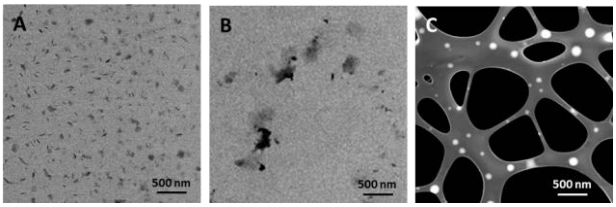

FIG. 1. Nanoparticles of bioactive substances (A-fluorouracil, B- carbenicilin, C- gentamicin) synthesised via one step sonochemical method.

Conclusions

MD simulations and experiments were conducted to unravel the mechanism of sonochemical nanoparticles formation of drugs. MD simulations demonstrated to be a suitable technique to investigate the molecular approach for drug nanoparticles formation. The presence of phase boundary of ethanol and water at the bubble interface place an essential role in the nucleation and further growth of drug nanoparticles. The proposed strategy for molecular simulation is simple and can be easily transformed for the investigation of nanoparticles formation of other drug molecules and in the broader perspective also for deposition of nanoparticles over the polymeric biomaterials surfaces.

Acknowledgments

This study was financed by the Polish National Science Centre project awarded by decision number 2021/03/Y/ST4/00071. This research was supported in part by PLGrid Infrastructure.

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POLYNIPAAM AND FUNCTIONALIZED MWCNTs AS COMPONENTS OF BIOINK

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Introduction

The biofabrication of biomimetic tissue analogs, has enormous potential for research in organ physiology, pathology, cancer research and regenerative medicine. Bioprinting is one of the dynamically rising techniques to biofabricate biomimetic tissues, based on cellular self-assembly and -organization. In this technique, the bioink acts as a carrier for cells intended for 3D bioprinting and determines the success and precision of the process and quality of the final products.

One of the strategies to improve the bulk and surface properties of bioink (typically made of gelatin) is the incorporation of biologically-relevant substances (e.g. carbon nanomaterials, polymeric microgels) as well as components which provide rheological properties. Such additives can be successfully used to improve the mechanical properties of the bioink, and additionally enrich the hybrid material with antibacterial activity.

Since raw carbon nanotubes (CNTs) are entirely hydrophobic, their applications as biomaterials' components require surface functionalization. The presence of functional groups enables the anchoring of molecules to carbon surfaces, recently, the topic is intensively explored. Functionalization can either be covalent or non-covalent and the strategy applied depends mostly on the target application. Polar oxygen groups due to the electronegativity difference between carbon and oxygen atoms affect mostly the wettability of the carbon surface and its electron-donor properties. These features are often taken into account when designing carbon materials for specific applications, especially in the context of biomaterials and bioink in particular.

The aim of the study was to prepare a hybrid gelma-based bioink fuctionalized with MWCNTs and poly-NiPAAm additives to provide activity against bacteria.

Materials and Methods

Methacrylamide-modified gelatin (GelMA) was prepared following the protocol [1], in the studies, 10 w/v% GelMA hydrogel in PBS was used. MWCNTs (NanoAmor) used in the study were functionalized with oxygen plasma system (Diener Electronics). The modification was followed with the work function measurements (Kelvin Prove, McAllister). Poly-NiPAAm was prepared following the protocol [2]. The bioink was obtained with the use of ultrasound irritation (Q500 Sonicator). Final material was testes against *S. aureus* and *P. aeruginosa*) as control sample, solidified GelMa was used.

Results and Discussion

The oxygen plasma modification of MWCNTs was followed by work function changes ($\Delta\Phi$). It was found that the largest changes $\Delta\Phi$=1.2 eV were measured for MWCNTs modified with the following set of parameters p=0.2 mbar, P=100 W, t=6 s. Such changes in work function clearly indicate the introduction of surface oxygen groups which provide the electrostatic barrier for the electron transfer at the interface.

For the dispersion of oxygen plasma functionalized MWCNTs and poly-NiPAAm in the gelatin matrix, several sonication parameters were tested. It was found that the 100 W and 30s is sufficient to prepare well-dispersed suspension without overheating and the thermal damaging of the bioink. The parent as well as hybrid materials GelMa+MWCNTs+poly-NiPAAm were prepared in 24-well plates for microbiological studies. The schematic presentation of the prepared hybrid bioink material is shown in FIG. 1. For samples GelMa+poly-NiPAAm inhibition of growth was observed for *S. aureus*, while no effect for *P. aeruginosa*. Addition of MWCNTs significantly inhibited the growth of both bacteria strains *S. aureus* as well as *P. aeruginosa*.

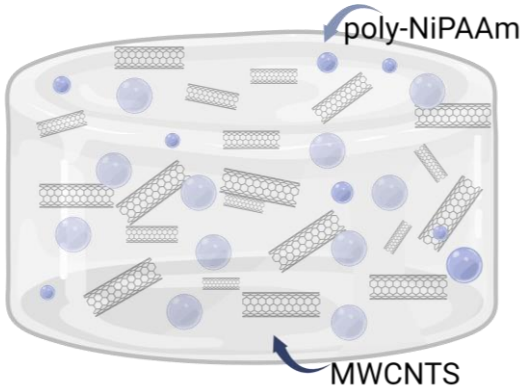

FIG. 1. Schematic presentation of the functionalized Gelma-based bioink showing the main components of the hybrid material.

Conclusions

It was found that oxygen plasma treatment of MWCNTs increase their hydrophilicity and therefore allows to obtain well-dispersed gelatin-based bioink. The GelMa+poly-NiPAAm samples effectively inhibit growth of Gram-positive *S. aureus*, while addition of MWCNTs broaden the spectrum also against Gram-negative *P. aeruginosa.* The results clearly show the additive effect of the two components used in this study.

Acknowledgments

This study was financed by the CELSA/20/020 SMARt biomaterials for bioprinting of vascularized TISsues (SMARTIS).

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GENERAL AND BROAD APPLICABLE METHOD FOR FUNCTIONALIZATION OF GELATIN

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Introduction

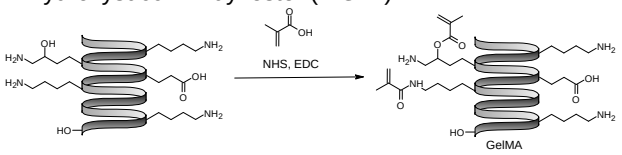
Gelatin is a naturally occurring polypeptide soluble in water. The content of individual amino acids and their order in the peptide chain depends on the origin of the gelatin.

Amino acids such as lysine, 5-hydroxylysine, proline, and 3-hydroxyproline play a crucial role in gelatin functionalization. Due to their presence, the peptide chain is rich in primary amine groups and hydroxyl groups, which are involved in nucleophilic substitution reactions with the appropriate acid anhydride or activated carboxylic acid.

A common method for modifying gelatin is to react the gelatin with an excess of the corresponding acid anhydride under aqueous conditions. An alternative method for introducing any acyl moieties into the gelatin structure is to use an activated carboxylic acid. Activation of the acid carboxyl group is accomplished by forming active esters which are susceptible to attack by nucleophiles such as amino and hydroxyl groups. For this purpose, the reaction uses a coupling reagent, such as EDC or DCC, and proper additives such as N-hydroxysuccinimide, 1-hydroxy-1H-benzotriazole or 1-hydroxy-7-azabenzotriazole.

By regulating the amount of active esters used in the reaction, it is possible to control the degree of substitution in the range from 20 to 100%, and thus the properties of the obtained product. It is also possible to introduce several different groups into one chain.

The most commonly used polymer is methacrylated gelatin (GelMA) [1,2,3]. It is formed by the reaction of free amino and hydroxyl groups present in the gelatin structure with methacrylic acid anhydride or a suitable active ester of methacrylic acid, e.g. N-hydroxysuccinimidyl ester (FIG. 1).



Modified gelatin is a photocurable and biocompatible polymer. It is an important substrate for the creation of materials used in tissue engineering and regenerative medicine.

Results and Discussion

Reaction of methacrylic acid with coupling agent such as EDC and DCC produces an active intermediate which then reacts with N-hydroxysuccinimidyl additive. As a result, the active ester of methacrylic acid is formed. Only this active ester is reacted with gelatin.

The active ester is relatively stable under water conditions. Nevertheless, literature data indicate that the stability of N-hydroxysuccinimidyl esters is pH dependent. The lower the pH, the more stable the ester. The most favorable pH is about 4.5-7.2. On the other hand, the reaction of the active ester with amino groups is most effective at pH 7-8. In addition to the pH, the solvent used, the type of an active ester, the type of a coupling reagent, the excess of ester in relation to the amine groups as well as the time and temperature of the reaction have an impact on the efficiency of the reaction and degree of substitution.

Conclusions

This method allows any substituents to be attached to the gelatin. The only condition is that at least one carboxyl group should be present in the substituent structure. Thanks to this method, it is possible to reduce the production of harmful by-products and to carry out the reaction efficiently with acids whose anhydrides are not commercially available. The method can be used to attach biologically active compounds such as vitamins, antibiotics, drugs to potentially create their carriers that gradually release these therapeutics. Moreover, thanks to the use of compounds activating the carboxyl group, it is also possible to effectively carry out modifications to other biopolymers.

Acknowledgments

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

Conflicts of Interest

Michal Wszoła is the co-founder of Polbionica Ltd.

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NEW ASPECTS IN LACTOFERRIN STUDY

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Introduction

LTF is a multifunctional metalloprotein which reveals antibacterial, antiviral, antifungal, anti-inflammatory properties. Moreover, it takes part in immunomodulation, immunoregulation processes as well as regulates cell migration and proliferation. Lately, the utilization of LTF is considered as the active substance in wound healing preparations. However, it noteworthy to mention that LTF biological activity is highly dependent on its physicochemical properties. Depending on the iron-saturation status LTF is divided on holo- and apo-LTF. Instead, according to glycosylation level LTF-a and LTF-b can be distinguished. Apo-LTF due to its iron-sequestering properties has shown to have higher antibacterial properties as well as LTF-b which has higher stability [1]. Moreover, it was identified at least 59 distinct *N*-glycans in LTF which also has shown to have impact on biological and physicochemical properties of the protein [2]. Thus, the aim of the study was to develop methodology for the LTF characterization.

Materials and Methods

Bovine LTF (bLTF) standard (≥85%) supplied from Sigma-Aldrich (Steinheim, Germany) was utilized in the study. The SDS-PAGE coupled with MALDI-TOF/TOF-MS was utilized. The SDS-PAGE was carried out according to the standard protocol recommended by the manufacturer (Bolt™, ThermoFisher Scientific). In-gel tryptic digestion with subsequent MALDI-TOF/TOF-MS analysis was performed as described in P. Pomastowski et al. [3]. MALDI-TOF-MS analysis in linear mode was performed according to procedure provided by equipment manufacturer (Bruker Daltonics) with utilization of Sinapinic acid (SA) as a matrix. Spectra records were performed on MALDI-TOF/TOF mass spectrometer UltrafleXtreme II (Bruker Daltonics). The zeta potential measurements were performed on a Malvern Zetasizer NanoZS (Malvern). The analysis was performed in different buffers, namely in sodium chloride solution as well as in 0.01 and 0.05 M ammonium citrate buffers [3]. Finally, for ICP-MS analysis protein mineralization in 65% HNO_3 during 3 h at 80°C was performed. The analysis was carried out on Shimadzu ICP-MS 2030 with scandium as an internal standard.

Results and Discussion

SDS-PAGE revealed numerous bands of proteins in the range of 14 to 198 kDa indicating the presence of impurities in the protein. The supplier declared the purity of the standard to be not less than 85% by SDS-PAGE assay. The presence of impurities may come from the imperfection of isolation method or as a consequence of protein degradation [4,5]. Thus, to identify detected impurities we performed in-gel digestion and registered the spectra of obtained samples with MALDI-TOF/TOF-MS. Subsequently, the analysis of spectra was performed with ProteinScape software (Bruker Daltonics) by applying Mascot search. The results showed that almost all studied proteins were identified as bLTF in mass range between 62 and 98 kDa as well as its dimer and fragments, but the protein with a mass of nearly 14 kDa was shown to be bovine keratin.

SDS-PAGE is usually utilized for the determination of protein molecular mass. However, to determine the exact protein mass the protein markers of proteins with very small differences should be utilized. Instead, MALDI-TOF-MS in linear mode enables to estimate the molecular weight of bLTF ($[M+H]^+$). Our study showed, that average masses of analysed bLTF were in the range of 82 to 84 kDa with a maximum ≈ 83.200 Da. While supplier declared the protein mass ≈ 87 kDa. Moreover, in the previous study of our group, the average masses of isolated bLTF were in the range of 77 to 81 kDa with a maximum of 77.700 Da [3]. bLTF is the glycoprotein and the differences in masses are related to its glycosylation degree. bLTF has 5 possible glycosylation sites and 4 of them are always glycosylated (Asn233, 368, 476, and 545), whereas the fifth (Asn281) is glycosylated in about 15 to 30% depending on the stage of lactation. The level of bLTF glycosylation is also dependent on other factors such as inflammation, environmental, and stress conditions [1,2]. It is noteworthy to mention, that biological activity of bLTF is dependent on its glycosylation state which emphasize the necessity to determine exact molecular mass of the protein before other studies. The spectrum of protein revealed also other signals which suggested to be multiple-charged bLTF ions and the impurities in the sample.

Zeta potential measurements was utilized for the determination of isoelectric point (pI) of bLTF. Literature data indicate that bLTF is highly cationic protein and has pI in the range between 8.0 and 9.0 [1]. However, our study showed that studied bLTF has net-charge equals 0 at pH 7.4 ± 0.2 when measurements were performed in 0,09 % solution of NaCl. The previous investigation performed in our group for bLTF with molecular weight ≈78 kDa has revealed a pI value of 6.0 ± 0.3 in the same conditions [3] which may be connected with the different glycosylation, among others, the presence of higher amount of sialic acid groups [2]. Interestingly, the presence of citrate ions in the solution shifted the pI to lower values. The pI for bLTF in 0.01 M and 0.05 M ammonium citrate was established at 5.5 and 5.0 respectively.

Finally, ICP-MS analysis revealed that each bLTF molecule contains two Fe^{3+}. Thus, it can be concluded that bLTF supplied by Sigma-Aldrich is in holo-form which should decrease antibacterial activity of the protein.

Conclusions

The study aimed the development of methodology for bLTF study. The results indicate that depending on utilized technique the same parameter may differ significantly which should be considered in the study of biological activity of bLTF and its utilization in pharmaceutical preparations.

Acknowledgments

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2nd SKIN HYDROGEL DRESSING MATERIAL AS A FACE MASK AFTER INVASIVE COSMETIC AND AESTHETIC MEDICINE PROCEDURES

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Introduction

There is a great variety of dressing materials for the management of acute and chronic wounds. However, it is still challenging task to create an ideal biomaterial that would provide optimal conditions for the effective regeneration process. Non-surgical cosmetic procedures, in particular that focusing on the face area, are one of the most intensively developing branches of beauty industry. Invasive procedures, such as microdermabrasion, platelet-rich plasma therapy, needle mesotherapy, or medical peels, require skin regeneration and convalescence. The 2nd skin hydrogel dressing releasing pro-healing agents, prepared in the form of thin and elastic film, may serve as an excellent face mask for the convalescence and skin revitalization after invasive cosmetic procedures.
The aim of this study was to prepare hydrogel thin film using a blend of two biopolymers (chitosan and curdlan) and to determine basic biological properties of the resultant biomaterial.

Materials and Methods

Chitosan/curdlan thin film was produced based on the procedure described in a Polish patent application no. P.430456 (2019). Briefly, chitosan solution prepared in acetic acid was mixed with curdlan suspension in water. The blend was spread on the flat mold and subjected to thermal gelation at high temperature of 95°C.
Cytotoxicity of the biomaterial was assessed using human skin fibroblasts (BJ cell line, ATCC) in accordance with the ISO 10993-5 (2009) standard. Cell viability was determined by the MTT colorimetric test after 24 and 48 hours of incubation with the dressing extract.
The possibility of cell adhesion and growth on the surface of the hydrogel dressing was assessed by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). For CLSM observation fibroblasts were seeded directly on the biomaterial and stained with LIVE/DEAD fluorescent kit. For SEM observation cell-seeded samples were fixed in 3.7% paraformaldehyde, dehydrated in graded ethanol concentrations, dried and sputtered with a thin gold layer of 8 nm [1]. Cell adhesion and spreading on the surface of chitosan/curdlan film were evaluated after 48-hour culture of fibroblasts.

Results and Discussion

MTT cytotoxicity test showed that cell viability was approx. 100% after 24- and 48-h incubation in the presence of the chitosan/curdlan extract. Thus, dressing material was proven to be nontoxic to human skin fibroblasts. Nontoxicity of the hydrogel film was confirmed by LIVE/DEAD staining as only viable cells were detected on the surface of the material (FIG. 1). Both CLSM and SEM observation showed that hydrogel dressing allowed for good adhesion and growth of skin fibroblasts on its surface (FIG. 1). Thus, hydrogel dressing material may also act as skin substitute seeded with patient cells. It should be noted that skin substitute should primary provide an optimal microenvironment for skin cell survival, adhesion, and proliferation [1,2].

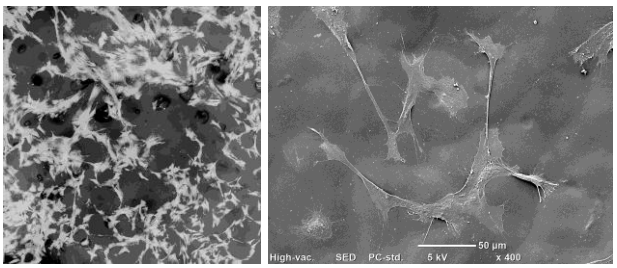

FIG. 1. LIVE/DEAD staining presenting viable and well attached fibroblasts (left image) and SEM image (on the right) of human skin fibroblasts grown on the 2nd skin hydrogel dressing.

Conclusions

The 2nd skin hydrogel dressing may be used as thin and flexible face mask enriched with bioactive agents to cover superficial wounds after invasive cosmetic procedures and to accelerate skin regeneration (FIG. 2). The 2nd skin may also be seeded with patient skin cells or stem cells and used as skin substitute in the case of more serious wounds (FIG. 3). Stem cells attached to the film will release pro-healing and anti-inflammatory factors, accelerating regeneration and reducing scarring.

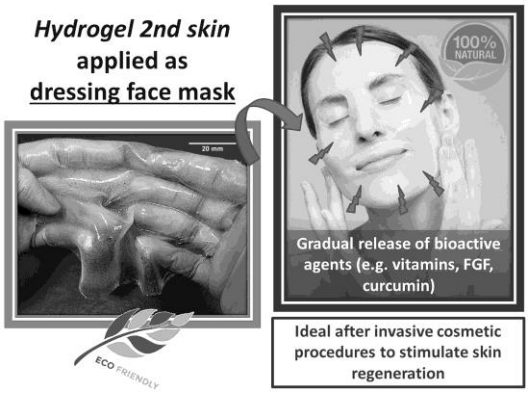

FIG. 2. The 2nd skin hydrogel dressing applied as a face mask enriched with bioactive agents.

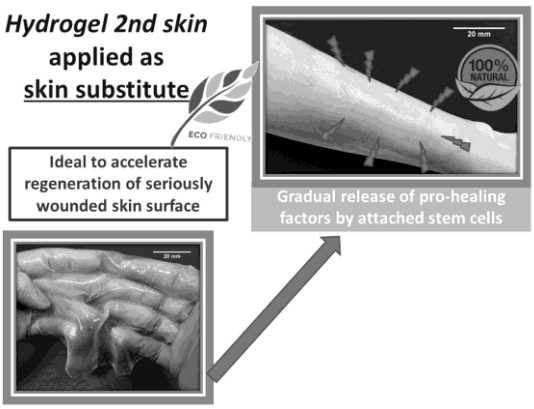

FIG. 3. The 2nd skin hydrogel dressing applied as a skin substitute seeded with stem cells.

Acknowledgments

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THE COMPARISON OF THE MAO COATINGS CHARACTERISTICS ON SLM-PRODUCED VS COMMERCIALLY AVAILABLE Ti13Zr13Nb ALLOY FOR BIOMEDICAL APPLICATIONS

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Introduction

Titanium alloys dedicated to biomedical applications require surface treatment to improve their bioactivity and some mechanical properties.
Among the surface modification methods, micro-arc oxidation (MAO) has gained special attention due to its capability to produce dense oxide film that binds well to substrate, enhances biocompatibility and bioactivity, coat the complex-shape objects, and can incorporate ions into the structure [1]. According to the literature, the MAO coating characteristics is closely related to the microstructure of the substrate. Different manufacturing methods with various thermal gradients and solidification result in different structure and grain boundary interfacial areas [2]. Since the progress in the development of implantology aims to use personalized implants, the surface modification of 3D printed materials should be elaborated in more detail [3].
The main aim of this work was to investigate the effect of the manufacturing method of the substrate on MAO coating characteristics.

Materials and Methods

Two groups of samples in the shape of disks were prepared from the: i) commercially available Ti13Zr13Nb alloy rod (Xi'an SAITE Metal Materials Development), and ii) selective laser melted (SLM 100, Realizer GmbH) Ti13Zr13Nb powder (TLS Technik GmbH & Co. Spezialpulver KG). The MAO process was performed using DC power supplies (MR100020, &K Precision Corp.) under constant process parameters: 400 V, 15 min, 216 A/m^2 in an electrolytic solution containing 0.1 M of calcium glycerophosphate (GP), 0.15 M of calcium acetate (CA) and 0.006 M of silver nitrate $AgNO_3$.
To obtain the characteristics of coatings, the microstructure (SEM; JSM-7800F), topography and surface roughness (AFM; NaniteAFM, Nanosurf AG), thickness (thickness meter; FMP10-20, Helmut Fischer GmbH), elemental composition (EDS; S-3400NX, Hitachi), crystal structure (XRD; Bruker D8 discover), chemical composition (FTIR; Perkin Elmer Frontier), and surface wettability (optical tensiometer; Attention Theta Life) were evaluated. The nanomechanical properties were determined using a nanoindenter, while MAO coating adhesion was estimated by the scratch test (NanoTest Vantage, Micro Materials Ltd).
The corrosion behaviour was characterised with a potentiostat/galvanostat (Atlas 0531, Atlas Sollich). The experiments on cytotoxicity were conducted on hFOB 1.19 cell line by the MTT cytotoxicity test and LDH release assay.

Results and Discussion

In all cases, porous, Ca- P- and Ag-containing titania-based films were successfully formed on the Ti13Zr13Nb alloy samples. The significant differences in microstructure between rod and SLM could be noticed (FIG. 1). Samples obtained by SLM were characterized by higher mechanical properties, lower hydrophilicity, and lower corrosion resistance compared to the commercial rod. MAO layers obtained on SLM specimens had smaller pores, greater thicknesses, and higher contents of calcium, phosphorus, and silver ions.

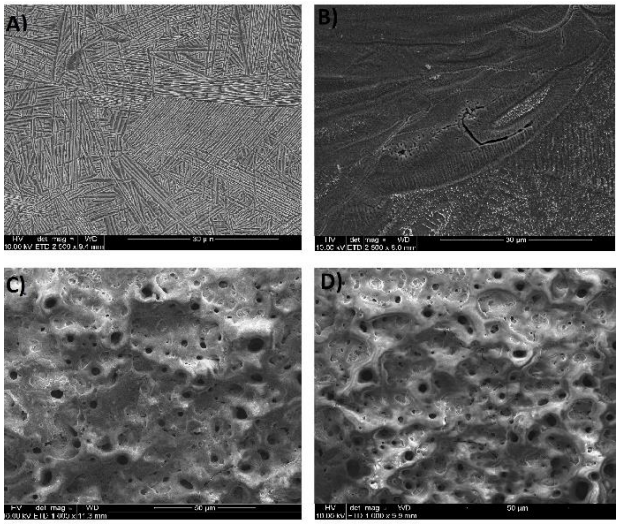

FIG. 1. Microstructure of: A) rod, B) SLM, C) MAO on rod, D) MAO on SLM specimens.

In all cases, the MAO coatings improved the wettability, mechanical properties, and corrosion resistance of Ti13Zr13Nb alloy. There were no significant differences between cell proliferation in all samples. Although the inhibition of the cell proliferation ratio was estimated around 40%, the MAO coatings did not lead to cell necrosis because the LDH release was not aggravated in the tested cells.

Conclusions

The results indicate that surface treatment of titanium alloy is crucial for biomedical application. The MAO coatings characteristics are strictly related on the manufacturing method of the substrate. The differences in microstructure between commercially available rod and selective laser melted specimens have a significant effect on morphological, physical, chemical and mechanical properties of MAO coatings. The combination of SLM and MAO strategy is the perspective method to improve properties of titanium alloys for biomedical application and further investigation should be conducted.

Acknowledgments

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PROPERTIES OF NEW COMPOSITE MATERIALS BASED ON HYDROXYAPATITE AND CROSS-LINKED GELATIN FOR BIOMEDICAL APPLICATION

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Introduction

Although there are many solutions for replacing bone structures or fixing titanium implants to the bone, there is still little research focused on obtaining quick-setting composites that guarantee immediate primary implant stabilization [1]. The main aim of the research was to develop a new biocompatible and injectable composite with the potential for applications as the bone-to-implant bonding material or as a bone substitute. The new formulation and fabrication method were evaluated. The composite based on hydroxyapatite, gelatin, and two various types of transglutaminase (TgBDF/TgSNF), as a cross-linking agent, was proposed. The impacts of composite content and process parameters on various properties of the material were assessed.

Materials and Methods

To prepare the composite, 4 g of gelatin was dissolved in 9 mL of distilled water at 50°C and stirred with a magnetic stirrer at 200 rpm for 5 min. Then 8 g of hydroxyapatite powder with an average grain size of <200 μm (Sigma Aldrich) was added while stirring continuously at 50°C. Two types of transglutaminase, namely BDF (BDF Natural Ingredients S.L) and SNF (TG-S-NF, Ajinomoto Co.), which had previously been dissolved in 1 mL of distilled water, were added to make a composite paste at 5, 10 or 15 wt.%. (per weight of gelatin). After 10 s of intensive mixing, the composite was placed in properly prepared polymer molds. After curing, the samples were removed from the molds and left for 5 days at room temperature to evaporate water. The following research was performed: 1) the injectability – measurement of the max. application time of the paste by injection until its hardening with automatic injector; 2) the morphology by SEM microscopy (FEI Quanta FEG 250); 3) the chemical structure by FTIR spectroscopy (PerkinElmer Frontier); 4) the surface wettability by optical tensiometer (Attention Theta Life); 5) the degradation behaviour after 1, 3 and 5 days in simulated body fluid (PBS solution); 6) the mechanical properties using Olivier-Pharr indentation analysis (NanoTest Vantage); 7) the cytocompatibility – assessment of human osteoblast viability (ATTC-CRL-11372) by MTT and LDH assay.

Results and Discussion

With concentration increasing from 5 to 15% of TgBDF the injectability decreased about 58% and reached an optimum in 8 minutes. In the case of TgSNF, no significant differences were notable, and injectability was obtained in ~11 min for all contents. Based on SEM images, no difference in the surface morphology among the specimens was noticed. FTIR spectra indicated that the cross-linking effect did not significantly affect the chemical structure. In addition to the peaks specific to gelatin and hydroxyapatite, another peak was found which indicating the formation of a new bond Ca-COO- (~1345 cm^{-1}) [2] confirming the proper mixing of both components. The contact angle measurements showed that the composites contained TgBDF were characterized by better hydrophilic properties compared to those with TgSNF. The degradation behaviour was higher for composite with TgBDF (91-93%) compared to TgSNF (77-87%) after 24 h in PBS solution and increased with immersion time. All samples demonstrated very prompt nanomechanical properties with Young modulus and hardness close to the values of the natural bone, 10.03±0.90 and 0.27±0.01 GPa, respectively (FIG. 1) [3].

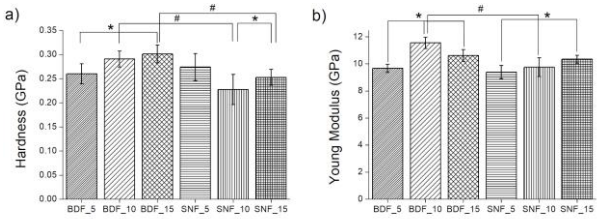

FIG. 1. Nanomechanical properties: (a) hardness; (b) Young's modulus for composite materials (n=10; *significantly different by transglutaminase concentration, #significantly different by transglutaminase' type).

Culture of the cells with BDF/SNF for 24 h did not cause cell death. The SNF at concentrations of 5% and 10% did not lead to cell necrosis because the LDH release rate was not meaningly enhanced in the tested cells. Moreover, the cell morphology was not characterized by the necrotic phenotype. On the other hand, cellular proliferation was significantly inhibited, because the MTT reduction rate was about 40% in these conditions. An increase in this transglutaminase in the composite to 15% increased LDH release by 33% but the cellular proliferation was about 62% under these conditions. The cell number decreased by only 20% with 15% SNF.

Conclusions

The type of transglutaminase did not significantly affect the surface topography, chemical composition, or application properties such as injectability. Regardless of the cross-linking agent, all samples demonstrated very good nanomechanical properties, with Young's modulus and hardness close to the values of natural bone. The cytotoxicity studies proved the influence of the type and rate of transglutaminase on the cellular response. In all cases, the transglutaminase did not lead to cell necrosis, but cellular proliferation was significantly inhibited, especially by BDF.

Acknowledgments

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THE MICRO-INJECTION MOULDING OF BIODEGRADABLE POLYESTERS IN THE COURSE OF THE VASCULAR STENT MANUFACTURING PROCESS

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Introduction

Biodegradable polyesters are very good materials for the construction of medical implants, due to their biocompatibility, bioresorbability and non-toxicity [1,2]. Cardiovascular implants, such as stents, are characterized by a thin-wall, openwork construction with a high degree of complexity (FIG. 1).

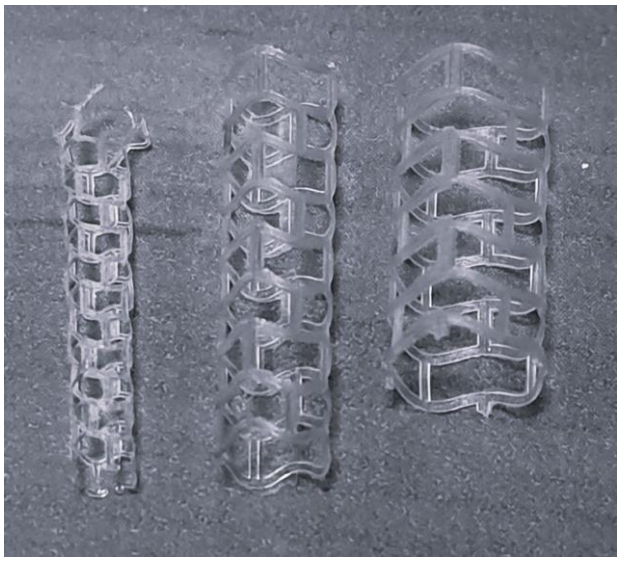

FIG. 1. Series of biodegradable vascular stents in different diameters (3,0 mm, 5,4 mm and 7,4 mm respectively) obtained by micro-injection moulding.

For this reason, in the processing equipment, a special injection unit, adapted to micro-injection moulding process is usually used. It is characterized by a two-stage plasticizing system. Picking and pre-plasticizing of the material takes place by a screw, that doses molten polymer to the injection cylinder. Injection to the mould is carried out by the injection plunger (high injection precision).

Processing of biodegradable polyesters on an industrial scale poses a challenge, due to thermal degradation [3,4]. Relatively poor thermostability, results in quite narrow range of processing parameters. Despite processing difficulties, such a materials are the subject of intensive scientific research [5]. It is necessary to place a strong emphasis on process optimization, so that the choice of parameters allows to obtain a moulding with the desired properties.

The aim of the research was to optimize the parameters of the micro-injection process of biodegradable polyesters in order to eliminate defects and obtain the high-quality mouldings.

Materials and Methods

The polymeric material was processed using a MicroPower 15 micro-injection moulding machine (Wittmann Battenfeld). The mould was thermostated with water. The quality of stents was observed with a stereoscopic microscope working in polarized light (DeltaOptical), digital microscope (Keyence) and scanning electron mictroscopy (SEM) (FEI Company). The mechanical properties were measured on the crimper device (tensile test machine from Blockwise Engineering) intended for measuring radial forces.

Results and Discussion

During a series of tests, the injection parameters of the micro-injection molding process, such as: volumetric melt stream, mould cavity temperature (both during injection and de-moulding), melt temperature, injection pressure, packing pressure and time, were optimized. In order to obtain a appropriate quality of the mouldings, it was necessary to use a thermostat with 2 medium circuits. This allowed for injection at the processing temperature of a given material, and then cooling the mold cavity to a temperature enabling the de-moulding of the stent without deformation. The use of electric heaters in conjunction with water cooling was not possible due to insufficient space around the mold cavity due to the very small dimensions of the components obtained. When evaluating received mouldings, following criteria were taken into account: correct representation of the geometry of the stent, without defects and de-moulding without damaging the structure.

Conclusions

It has been proven, that varying the injection parameters, mouldings of desired shape without defects were obtained, which is reflected in the mechanical properties of the obtained implants.

Acknowledgments

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Thanks to Keyence for providing a VHX-7000 digital microscope for imaging of the received stents and their quality control.

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COMPARISON OF PERIODICAL AND CONTINUOUS PROCESSES OF RING OPENING COPOLYMERIZATION OF L-LACTIDE AND GLYCOLIDE IN BULK

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Introduction

During the course of polymerization reaction conducted in bulk, the viscosity of the reaction volume begins to increase as the degree of the monomer conversion increases. This hinders diffusion processes and heat exchange, which can lead to inhomogeneous location of the initiator as well as local overheating, even resulting in thermal degradation of polymer chains. Diffusion and heat conduction processes are gaining more and more importance along with the increase in the volume of the reaction mass. Therefore, mixing becomes crucial, as it homogenizes the reaction mixture and equalizes the temperature, and also lowers the viscosity due to the action of shear forces.

Poly(latcide-co-glycolide) (PLGA) due to its biodegradability and biocompatibility is one of the best studied polymer biomaterial which also has been approved by FDA [1,2]. For this reason its use has become increasingly popular in many fields of science. Fused Deposition Modelling (FDM) 3D printing is an additive manufacturing method which enables the rapid production of biodegradable implants and tissue engineering scaffolds [3]. The popularization of additive manufacturing resulted in the development of the polymer filaments market, but the availability of certified and tailored materials for medical applications is still insufficient in this field.

In present work, a comparison of three methods of obtaining PLGA copolyester in bulk, with the use of a non-toxic initiator in the form of zirconium (IV) acetylacetonate was carried out. It was a step towards increasing the synthesis efficiency in order to scale up the production of commercial medical-grade 3D printing filament.

Materials and Methods

Poly(L-lactide-co-glycolide) copolymer was synthesized via ring-opening copolymerization (ROP) of L-lactide and glycolide (both HUIZHOU Foryou Medical Devices Co., Ltd., China) in the presence of a $Zr(acac)_4$ initiator (Sigma Aldrich, Merck KGaA, Germany) [4]. Two compositions were obtained, differing in the molar ratio of lactidyl and glycolidyl units (85:15 and 50:50). The synthesis was carried out using three different devices: a PFA reactor, industrial mixer (30EHT 3Z, Brabender, Germany) and twin screw extruder (TSE 20/40, Brabender, Germany). A comparison of a standard procedure performed in a PFA reactor to a synthesis using industrial processing equipment was carried out. Reactions were conducted at two temperatures: 130°C and 160°C for 24 h. The progress of the polymerization reaction was examined by Nuclear Magnetic Resonance spectroscopy (Bruker Avance II Ultrashield Plus, USA). Monomer conversion, copolyester composition and microstructure were determined from the proton and carbon spectra. Gel Permeation Chromatography (Spectra Physics SP 8800 chromatograph, USA) was used to investigate average molar mass and Differential Scanning Calorimetry (Q2000 DSC, TA Instruments, USA) for study the thermal properties. The mechanical strength of synthesized copolyesters were investigated in the static tensile test (Instron, Model 4204, USA). Filaments for a 3D printer were also made in order to check whether it was possible to use them to make FDM prints.

Results and Discussion

The production of PLGA copolymer in a periodical process (PVA reactor and a mixer) and a continuous extrusion process were compared. A high conversion (99%) copolyester obtained in the PFA reactor was used as reference material, which was the standard method of production. Differences in the rate of the ring-opening polymerization reaction over 24 h were observed. This was reflected in obtaining various degrees of the monomer conversion, a different PLGA molar masses as well as chain microstructure. It also influenced the mechanical properties such as tensile strength and Young's modulus. It was observed that the change of the reaction temperature intensified the differences in the properties of the copolyester obtained by various equipment.

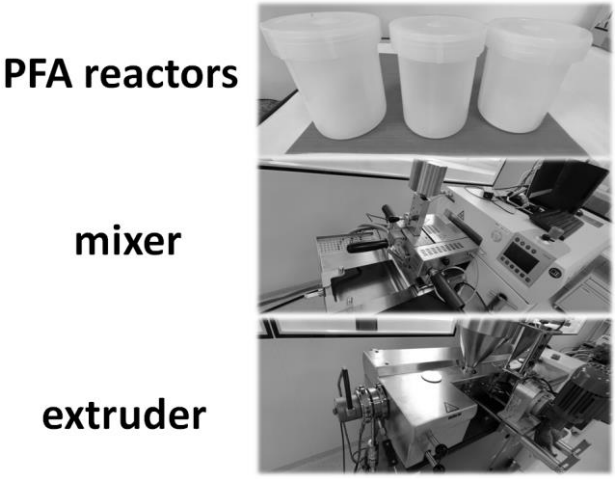

FIG. 1. Equipment used to synthesize PLGA copolyester.

Conclusions

Synthesis using industrial devices is possible, however, due to the lack of an inert gas atmosphere, lower molar masses of PLGA were obtained. The process therefore requires further optimization in terms of the amount of initiator used. On the other hand, by mixing the reaction mass, the polymerization was faster and the resulting polymer was more homogeneous.

Acknowledgments

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IMPROVED CYTOCOMPATIBILITY OF TiO_2 SCAFFOLDS BY SURFACE MODIFICATION WITH WHEY PROTEIN ISOLATE NANOFIBRILS

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Introduction

Bone defects arise as a result of trauma, tumour resection, osteoarthritis, etc., which significantly decrease the quality of patient life. Autografts are considered the gold standard for the treatment of bone defects. However, they show some limitations, which leads to the search for solutions among artificial scaffolds. Metals (cobalt or titanium alloys), polymers (collagen, chitosan, poly(lactide-co-glycolide), poly-ε-caprolactone), ceramics (TiO_2, hydroxyapatite), and their composites have been studied for their osteogenic properties and ability to support the formation of new functional bone [1]. Surface modification of the scaffolds with growth factors such as bone morphogenetic protein 2 (BMP-2) may enhance bone tissue healing. However, BMP-2 isolation and production are complicated and expensive. Furthermore, BMP-2 can cause some negative side effects, such as ectopic bone formation. The alternative may be the use the whey protein isolate (WPI), a waste product in the dairy industry, that consists mainly of β-lactoglobulin (β-LG). β-LG after heating for several hours in an acidic environment degrades to low molecular weight peptides that self-organize to form amyloid fibrils. The fibrils created on model glass surface have been shown to increase mesenchymal stem cell proliferation and osteogenic differentiation [2,3].
The purpose of this work was to develop a modification method of TiO_2 scaffolds with WPI nanofibrils, characterize their properties and analyse their performance in contact in model osteoblast-like cells.

Materials and Methods

TiO_2 scaffolds were manufactured using the polymer sponge replication method and sintered at 1500°C according to a previously developed method [4]. Scaffolds (height = 8.01 ± 0.08 mm, diameter = 8.89 ± 0.54 mm, weight = 99.22 ± 3.52 mg) were placed in a syringe, which contained 3 ml of 2.5% wt. The WPI solution was incubated at 37°C for one hour for surface modification purpose. The scaffolds were then triple rinsed in 2 ml of water at pH 2 and dried in air. The cytocompatibility of TiO_2 scaffolds was evaluated in contact with human osteoblast-like MG-63cells. Cells were cultured in modified Eagle's medium (MEM) supplemented with 10% of foetal bovine serum (FBS), 1% of antibiotics (penicillin-streptomycin mix), 0.01% of amino acids, and 0.01% of sodium pyruvate solution. Cells were seeded on the scaffolds at the initial density of 60,000 cells/scaffold and incubated in a humidified atmosphere at 37°C, with 5% CO_2. Cell viability was analysed using the resazurin reduction assay, live/dead, and haematoxylin/eosin staining after 1, 3 and 7 days. In addition, cells were observed with scanning electron microscopy (SEM) after fixation in paraformaldehyde and secondary fixation in osmium tetraoxide. Finally, the cells were dehydrated using the alcohol pathway and sputtered with a thin gold layer.

Results and Discussion

The results show that TiO_2 scaffolds (T) and TiO_2 scaffolds modified with WPI (T/WPI) are not cytotoxic for the cells. The metabolic activity assay showed that cell viability for the T/WPI scaffolds increases by approximately 20% compared to the T scaffold after 7 days (FIG. 1). These findings were confirmed by live/dead and haematoxylin/eosin staining, and by SEM images.

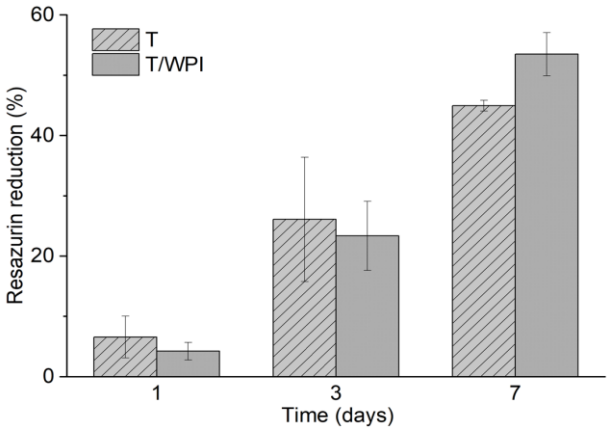

FIG. 1. The metabolic activity of MG-63 cells cultured on TiO_2 scaffolds: unmodified (T) and modified with WPI nanofibrils (T/WPI)

Conclusions

In summary, TiO_2 scaffolds can be modified with WPI nanofibrils. This modification improves bone cell growth and viability, so the material appears promising for the treatment of bone tissue defects. However, more experiments are necessary, such as osteogenic differentiation, mineralized nodule formation, alkaline phosphatase activity, collagen expression, etc. prior to in vivo studies and further clinical trials.

Acknowledgments

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MODIFICATION OF ZIRCONIUM OXIDE SURFACE IN ORDER TO OBTAIN INCREASED BIOACTIVITY AND ANTIBACTERIAL EFFECT

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Introduction

Bone infections are considered complicated problems and are associated with a high percentage of relapses [1]. Due to the poor distribution of antibiotics in bone [2] and bacterial biofilm formation [3], their treatment remains a worldwide challenge. Implants that exhibit antibacterial properties are considered a more efficient solution compared to conventional treatment. Coating ceramic substrates with calcium-phosphate (CaP) layers leads to improved bioactivity [3]. Moreover, the use of antibiotic-loaded polymer nanoparticles (NPs) in the layer allows an antibacterial effect to be achieved.
The aim of the study was to: i) bioactivate zirconia substrates with CaP layers; ii) manufacture polymer NPs; and iii) achieve the antibacterial effect of substrates through immobilization of NPs in the CaP coating.

Materials and Methods

Calcium-phosphate (CaP) layers were deposited on the ZrO_2 substrate by two-step immersion in a ten-times concentrated solution of simulated body fluid (SBF). Coated samples were tested using scanning electron microscopy (SEM) and X-ray diffraction (XRD). Antibiotic-loaded NPs were used to functionalize the surface of the covered substrate. To obtain NPs, the double emulsion method was used. The size and shape of NPs were checked by means of dynamic light scattering (DLS) and SEM, respectively. With the OPA assay, the encapsulation efficiency (EE) and drug loading (DL) were examined. Nanoparticles were immobilized in CaP layers by adding them into the SBF solution during second step of deposition. Drug release profiles were investigated by immersing samples in the phosphate-buffered saline (PBS) solution. The roughness of different surfaces was tested with a profilometer.

Results and Discussion

Microphotographs of samples after only the first step of coating (FIG. 1A) revealed that the surface is not fully covered, while samples after the entire process (FIG. 1B) exposed flakes-like crystals that form a layer on the entire surface. These preliminary conclusions were verified with the XRD analysis. According to the SEM pictures, crystals obtained during the second step are more densely distributed compared to those after the first step. The results of the profilometer measurements (TABLE 1) confirmed that the surface after the two-step deposition is less rough. Furthermore, the presence of NPs in the SBF solution has no negative impact on the crystallization process (FIG. 1C). Moreover, particles are present between the crystals (FIG. 1D) and appear to be attached to them.

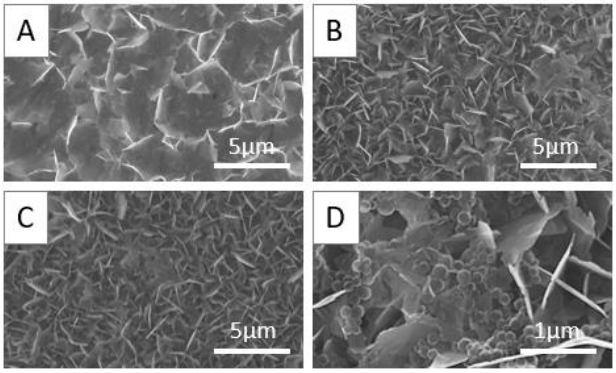

FIG. 1. SEM microphotographs of: A) coating after the first step of deposition; B) coating after the second step; C) coating after the second step with NPs incorporated; D) same as in fig. C with higher magnification.

TABLE 1. Results of the measurements of the profilometer of different surfaces.

	Ra ± SD [µm]	Rt ± SD [µm]	Rz ± SD [µm]
ZrO_2	0.04 ± 0.01	0.36 ± 0.24	0.33 ± 0.04
First step	0.22 ± 0.08	2.28 ± 0.82	1.30 ± 0.40
Second step	0.08 ± 0.02	1.07 ± 0.38	0.62 ± 0.24
Second step with NPs	0.12 ± 0.02	0.86 ± 0.07	0.72 ± 0.12

Obtained NPs are of round and regular shape and nanometric size that is around 200 nm for both empty and loaded NPs. SEM images and DLS results showed that the addition of antibiotic does not adversely affect the shape or size of NPs. EE and DL of antibiotic-loaded particles were calculated and were equal to 21.69% and 2.03%, respectively. The study of drug release showed that the trend is similar for both samples coated with a CaP layer doped with NPs and for the NPs themselves.

Conclusions

The presented results showed that the method of manufacturing the NPs is an effective way to entrap the drug in a polymer. The applied process of ceramic surface bioactivation and immobilization of NPs allowed to obtain CaP layers that are additionally functionalized to have an antibacterial effect with prolonged efficiency to prevent implant-related infections.

Acknowledgments

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HYDROGELS FORMED IN 3D PRINTED POLY(VINYL ALCOHOL) SACRIFICIAL MOLDS FOR BONE AND CARTILAGE TISSUE ENGINEERING

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Introduction

3D printed poly(vinyl alcohol) (PVA) templates to produce hydrogel scaffolds can be easily obtained using inexpensive and widely accessible fused deposition modeling (FDM) method. Water soluble PVA can be removed without a need to application of harmful organic solvents. However, hydrogen molding gelation should be fast enough to preserve molded shape before PVA is partially dissolved in water released from hydrogels. Hydrogel before glation should also have low viscosity to facilitate proper infiltration of narrow mold channels. Those requirements are not fulfilled by typical natural and synthetic hydrogels.

The aim of this study was to obtain optimal hydrogel composition to produce porous scaffold of designed geometry and biological properties suitable for bone or cartilage cells growth.

In our hydrogel mixutre gellan gum (GG) was used as fast gelling component whereas gelatin (Gel) was used to provide better cell adhesion and mechanical strength. As it is known pure gelatin without crosslinking is easily soluble in water and culture media thus cannot be used in unmodified state. We hypothesise that GG-Gel mixture of particular composition will provide better hydrogel stability combined with the ability to support cell growth.

Materials and Methods

Hydrogel was produced by mixing solutions of GG and Gel (from porcine skin) at 70°C and then cooling to room temperature (RT). Gelatin cross-linked with tannic acid (TA) was also tested. Different initial compositions were tested:

- (1) 0.7% GG+0.06% $CaCl_2$ (control),
- (2) 10% Gel (control),
- (3) 0.5% GG+0.06% $CaCl_2$ + 10% Gel,
- (4) 0.25% GG+0.06% $CaCl_2$ + 10% Gel,
- (5) 0.5% GG+0.06% $CaCl_2$ + 5% Gel + 0.25%TA,
- (6) 0.5% GG+0.06% $CaCl_2$ + 10% Gel + 1%TA

Initial viscosity and time of gelation were measured by reometry. After gelation cylindrical samples were formed (d = 8 mm, h = 2 mm). Stability of material was tested for 1 day by incubating in water and in DMEM culture medium. Material loss and swelling were determined by dry mass measurement. Samples (with additional 2 mg/ml alkaline phosphatase – ALP) were enzymaticaly mineralized by incubation in 1M calcium glycerophosphate (CaGP) for 3 days. FTIR spectra of mineralized and TA crosslinked materials were recorded. Heat-treatment by keeping dried samples at 60°C for 3 days was used as additional method of gelatin cross-linking.

Biological properties of selected materials were tested in vitro by seeding MG-63 cells on hydrogel materials. Cells after 7 days were visualized after live-dead staining. Vitality was measured by metabolic activity test based on resazurin reduction (Alamar Blue).

Results and Discussion

All of tested materials except pure gelatin (sample 2) and TA-crosslinked material (sample 6) were able to gelyfy within 1-2 min time window necessary to inflitrate the PVA templates. Sample 6 was too viscous and not optimal for molding. Therefore lower concentrations of Gel and TA were tested and proved to be suitable (sample 5) for molding. GG containing materials were dimentionaly stable in DMEM (except sample 5 in water), however 1% TA material was the only material completely stable in DMEM medium, whereas other materials were losing majority of gelatin component during 1 day incubation. This lost can be significantly reduced by alternative crosslinking involving heat treatment. Mineralization of hydrogels was only possible for composition non-crosslinked by TA as it was harmful for ALP enzymatic activity.

Biological test showed that crosslinking by 1% TA (sample 6) greatly increased cell attachment, whereas 0.25% TA (sample 5) did not. Mineralization do hydrogel was also increasing cell viability and attachment compared to non-mineralized counterparts. Lowering GG concentration (sample 4) led to inferior biological properties for mineralized samples (higher gelatin loss). Drying and heat treatment produced dimentionaly stable hydrogels, not soluble in water with biological properties similar to those crosslinked with TA.

Conclusions

Results indicate that optimal strategy to produce GG-Gel hydrogel scaffold molded in PVA templates is to apply:

- 0.5% GG – providing fast gelling
- 10% gelatin – providing biological clues
- use ALP mineralization approach to increase stiffness, thus produce material more suitable for bone tissue engineering
- use postcurring (in dry or liophylized form) by heat treatment providing additional crosslinking increasing gelatin stability in cell culture medium

Acknowledgments

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HYDROGELS BASED ON CHITOSAN WITH THE ADDITION OF STRONTIUM- AND ZINC-DOPED CALCIUM-RICH BIOACTIVE GLASSES

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Introduction

Hydrogel materials are well known for their beneficial properties such as great swelling, large water content, highly porous three-dimensional network structure, biocompatibility and the ability to mimic native extracellular matrix with the creation of friendly environment for cells. Hydrogels can be easily modified with biologically active components such as antibiotics, drugs or therapeutic ions, which will be released from their three-dimensional structure during gradual degradation of material. The source of therapeutic ions such as Sr^{2+} or Zn^{2+} can be bioactive glasses (BGs) obtained via sol-gel technique. Strontium ions play a key role in bone formation and regeneration, while zinc ions have antimicrobial properties and stimulate osteoblast differentiation, proliferation, and mineralization through the gene expression of various proteins. Thus, the introduction of BGs with the addition of therapeutic ions (Sr^{2+} and Zn^{2+}) acting as biologically active components into hydrogel materials can lead to the creation of multifunctional composite materials with properties extremely beneficial for tissue engineering.

Materials and Methods

The subject of this study are lyophilized hydrogel materials based on chitosan cross-linked with a functionalized dextran with the addition of calcium-rich bioactive glasses. Sol-gel-derived BGs such as A2 (40 mol% SiO_2, 54 mol% CaO, 6 mol% P_2O_5), strontium-doped A2Sr5 (40 mol% SiO_2, 49 mol% CaO, 6 mol% P_2O_5, 5 mol% SrO) and zinc-doped A2Zn5 (40 mol% SiO_2, 49 mol% CaO, 6 mol% P_2O_5, 5 mol% ZnO) act as functional components. The aim of this research was to evaluate the impact of the presence of different BGs on the physical, chemical and biological properties of chitosan-based hydrogels. All materials were incubated in PBS and SBF solutions in order to test their swelling, degradation and bioactivity. FTIR and SEM/EDS were used to evaluate structural and morphological changes of materials during incubation in SBF solution, while the ICP-OES analysis was held to evaluate the changes of ion concentration in SBF. Moreover a preliminary *in-vitro* studies of the biological response were carried out on Hs680 fibroblasts.

Results and Discussion

Results of this research showed that the presence of BGs doped with strontium and zinc influenced the properties of chitosan-based hydrogel materials – not only the structural, chemical and physical, but also biological properties tested *in vitro*. BGs significantly influenced the degradation (FIG. 1) of hydrogel materials and limited their swelling (FIG. 2). Moreover their presence contributed the improvement of bioactive properties of hydrogels and had a positive effect on Hs680 fibroblasts grown in direct contact with materials.

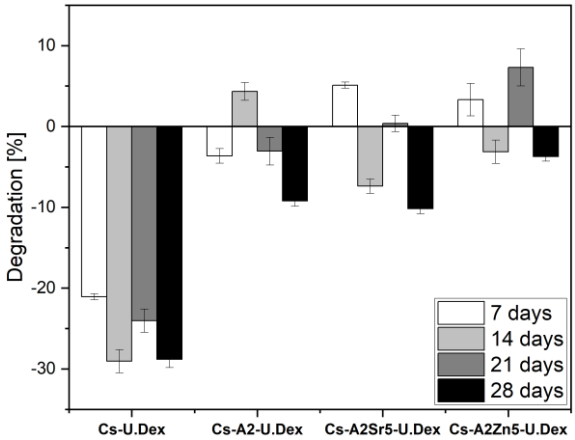

FIG. 1. Degradation of materials after 7, 4, 21 and 28 days of incubation in PBS solution.

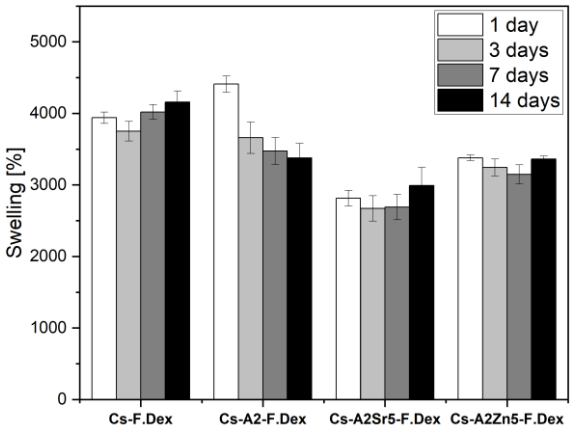

FIG. 2. Swelling of materials after 1, 3, 7 and 14 days of incubation in PBS solution.

Conclusions

Based on the conducted research, it was found that by appropriate selection of calcium-rich bioactive glasses it is possible to affect the physicochemical and biological properties of hydrogel materials. The obtained materials have promising multifunctional properties and great potential for use in tissue engineering.

Acknowledgments

This work was supported by the National Science Centre, Poland, grant nos. 2017/27/B/ST8/00195 (KCK), 2019/32/T/ST5/00453 (BZ) and program „Excellence initiative – research university" for the AGH University of Science and Technology.

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THE CHARACTERISTIC OF COMPOSITE COLLAGEN-MWCNTS FOILS OBTAINED FROM BENIGN DMSO/PBS-BASED SOLVENT

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Introduction

Collagen is a biopolymer abundantly found in the extracellular matrix (ECM) of mammalian tissues. Its morphology, chemistry, and mechanical properties aid in the adhesion, proliferation, and differentiation of cells, strongly influencing their behaviour. Therefore, it is actively being investigated as a biomaterial that can be used as a substrate in *in vitro* cell cultures [1,2]. Moreover, modification with nanomaterials, like surface-functionalized multi-walled carbon nanotubes (MWCNTs) can further improve the biological and physicochemical properties of the biopolymer [3,4]. Unfortunately, the processing of collagen is cumbersome, since it is a substance hard to dissolve. Due to this fact, toxic solvents like hexafluoroisopropanol (HFIP) are being commonly used [2]. It is of great importance to find alternative, benign solvents that would be able to maintain the native properties of the biopolymer. In our study, we propose a new type of solvent based on dimethyl sulfoxide (DMSO) and phosphate buffer saline (PBS).
The aim of this study was to obtain the composite collagen-MWCNTs foils from the DMSO/PBS solvent and compare their physicochemical properties to materials fabricated with the use of HFIP.

Materials and Methods

Oxidized (HO) and amidized (HNH) multi-walled carbon nanotubes used in this study were obtained by following the protocol established by Benko et al. [4]. Type I collagen (C9879), glycerol (G9012), hydrochloric acid (HCl), and fluoric acid (HF) were supplied by Sigma Aldrich. DMSO was bought from ChemPur, and HFIP was obtained from abcr company. Roti®-CELL PBS was supplied by Carl Roth.
In brief, 1% (w/v) collagen solutions were prepared by dissolving biopolymer in DMSO/PBS mixture (5:1) with the addition of 1 M HCl (0,1% $v/v_{DMSO/PBS}$) and 5% HF (0,1% $v/v_{DMSO/PBS}$). As a reference, 1% (w/v) collagen solution in HFIP was prepared. Mixtures were left on magnetic stirrers at 4°C for 12 days. After that, solutions based on DMSO/PBS were processed in a tissue homogenizer for 15 seconds. Next, all solutions were portioned, and to each sample, glycerol was added (5% $w/w_{collagen}$). Two samples of each kind were additionally modified with functionalized MWCNTs (0,25% $w/w_{collagen}$) of one type – HO or HNH. All components were mixed until fully homogenized mixtures were obtained. Solutions were poured on dishes made from PTFE and kept in the dryer at 37°C until fully dry. Samples were stored at 4°C until further use.
Chemical analysis of as-obtained samples was performed with the use of FTIR-ATR spectroscopy (Tensor 27, Bruker). Mechanical properties were evaluated with the use of Inspect Table universal testing machine (Hegewald-Peschke). Additionally, surface characterization analysis and degradation tests were performed on DMSO/PBS and HFIP-based materials.

Results and Conclusions

We managed to successfully obtain composite collagen-MWCNTs foils from both DMSO/PBS and HFIP-based solutions. The collagen maintained its native conformation in DMSO/PBS samples better than in HFIP-based materials. Physicochemical analysis of fabricated materials showed us that foils' properties were strongly dependent on the type of solvent used. The addition of glycerol and/or MWCNTs had also a significant impact on as-obtained materials.
Overall, DMSO/PBS/HCl/HF was found to be an excellent proposition as a new, benign solvent for the processing of commercially available collagen for potential use in the field of tissue engineering.

Acknowledgments

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The authors would like to acknowledge dr Ewa Stodolak-Zych for providing access to the tissue homogenizer used in this study.

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INHALABLE POLYMERIC CARRIERS OF TOBRAMYCIN AND CURCUMIN FOR THE TREATMENT OF BACTERIAL INFECTIONS IN THE RESPIRATORY SYSTEM

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Introduction

Treatment of bacterial respiratory infections, linked with the formation of biofilm is associated with many therapeutic challenges. Biofilm, which properties depend on the pathogen species; drug delivery systems specialized in targeting and / or penetrating a biofilm with high efficiency; and minimal side effects are only few of them [1]. Except that, the size of microparticles delivered by inhalation should be in the range of 1 to 5 μm [1,2].
The aim of this study was to obtain polyanhydride microparticles (MPs) carrying antibiotic tobramycin or a quorum sensing inhibitor (QSi) for inhalation delivery to treat bacterial infections of the respiratory system.

Materials and Methods

A polyanhydride copolymer of oligo(3-allyloxy-1,2-propylene succinate) (OSAGE) terminated with carboxyl groups and sebacic acid (SBA), in the ratios of OSAGE to SBA 40:60 (PSAGESBA 60) and 20:80 (PSAGESBA 80) were used as drug carriers. The tobramycin has been selected as an antibiotic and curcumin has been selected as a quorum sensing inhibitor (QSi). The particles had been produced by combining two techniques: double emulsification and freeze-drying. Isopropanol has been used as an emulsion stabilizer. The structure of the particles was evaluated using light microscopy, and the antibiotic concentration in the particles was assessed using o-phthalaldehyde (OPA) assay. To assess the quantity of encapsulated QSi three approaches were used: (1) assessment of curcumin in the supernatant; (2) particle dissolution in methanol; and (2) particle dissolution in dimethyl sulfoxide (DMSO). Furthermore, the Live-Dead and AlamarBlue tests were used to investigate how different concentration of MPs impacted human lung epithelial BEAS-2B cells growth and viability.

Results and Discussion

The obtained MPs varied from 0.5 to 5 μm in diameter, which corresponds to the values required for inhaled drug formulations reported in the literature [1,2]. The addition of isopropanol allows for more efficient and quicker tobramycin loading in the particles. From the obtained data it appears that higher loads of tobramycin can be obtained for PSAGESBA 80, therefore it can be concluded that the higher content of sebacic acid contributes to increasing the loads of antibiotic. However, despite the modification with isopropanol, the loading percentage was less than half the theoretical value, making it necessary to look for further method's improvements. The most efficient way of measuring loading for curcumin-containing particles was to dissolve the particles in DMSO. It enabled the full disintegration of MP and the assessment of the load content with the least dispersion. Higher loadings of curcumin could be produced with PSAGESBA 60. In vitro studies revealed that the concentration of MPs up to 100 μg/ml did not impact cell viability as compared to a reference, as shown in FIG. 1.

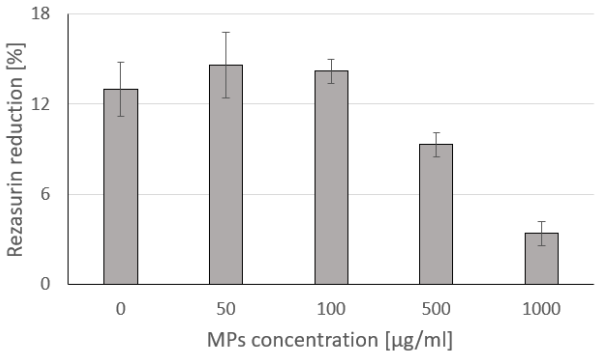

FIG. 1. The metabolic activity of BEAS-2B cells, dependent of MPs concentration.

Conclusions

It can be concluded that the higher sebacic acid concentration in the composition of polyanhydride copolymer provides for a higher loading in the MPs of the hydrophilic antibiotic tobramycin while lowering the loading of the hydrophobic QSi curcumin. MPs are cytocompatible with model lung epithelial cells.

Acknowledgments

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OPTIMIZATION OF THE CROSSLINKING PROCESS OF GELATIN/CHONDROITIN FIBRES AND GELATIN/ ALGINATE COMPOSITE SCAFFOLDS

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Introduction

Hydrogels are common materials useful in medical applications as scaffolds for tissue engineering or drug delivery systems [1,2]. They are natural polymers characterised by very good biocompatibility, high water absorption and degradation in the biological environment. Gelatin and calcium alginate blends are interesting examples of such materials. Gelatin is a water-soluble protein produced by the partial hydrolysis of collagen. The cross-linking process of gelatin increases its stability in the aquatic environment and allows to control the rate of its degradation. EDAC is one of the gelatin cross-linkers [2].

The aim of this work was to assess the impact of the crosslinking parameters, using EDAC reagent on the gelatin crosslinking process. Gelatin/alginate scaffolds modified with hydroxyapatite, nano-hydroxyapatite, and magnesium were tested. The crosslinking process of gelatin fibres was also optimised.

Materials and Methods

The following reagents were used: gelatin (Poch); alginate acid (Acros Organics); hydroxyapatite (Acros Organics); nano-hydroxyapatite (n-Gimat); $MgCl_2 \cdot 6H_2O$ (Poch); $CaCl_2$ (Poch); N-(3-dimetyloaminopropylo)-N′-etylokarbodiimidu, EDAC (Sigma-Aldrich); N-hydroxysuccinimide, NHS (Sigma-Aldrich); Phosphate Buffered Saline, PBS (Sigma-Aldrich). Composites were prepared by adding the modifier suspension (in distilled water) to the polymer solution to obtain the final concentration of hydrogels: gelatin 4.5 wt.% and alginate 1.5 wt.%. The solution with modifiers was ultrasonicated for 30 sec. The weight fraction of additives in composites was: 6% of HAp and 4% of magnesium chloride (GelAlg1); 6% of Hap, 4% of magnesium chloride, and 1% of nHAp (GelAlg2). The mixture of the polymer solution and the additives was poured into a mould and frozen in -20°C, 0.5 h. Next, the samples were cut into pieces (16 mm x 16 mm x 9 mm), and frozen in -80°C, 24 h. In the next step, the samples were freeze-dried for 48 h. Next, the samples were crosslinked in EDAC and $CaCl_2$ (1 wt.%, and 0.5 wt.% respectively) for 24 h and rinsed in distilled water (3 h). After that, the samples were frozen in -80°C, 24 h, and lyophilized again. The cross-linking process for the second group of samples was carried out before freeze-drying. The third group of the samples were gelatin fibres with chondroitin, crosslinked in the mixture of EDAC and NHS for 6 h (EDC/NHS ratio was 2.5:1). The fibres were obtained by electrospinning.

Infrared spectroscopy in ATR technique was carried out using the Bruker Tensor 27 spectrometer with diamond crystal. The materials microstructure was examined with the Keyence VHX-900F optical microscope. The *in vitro* degradation was carried out in PBS at 37°C for 2 weeks. Mass changes during the incubation were measured. The tests were triplicate. The standard deviation was used in the statistical analysis.

Results and Discussion

The microstructure of the scaffolds revealed the influence of the sequence of the performed activities in the process of their obtaining and crosslinking. The scaffolds which were crosslinked between freezing and freeze-drying showed about 5% less porosity than those crosslinked after the freeze-drying process. This may be caused by the material's contraction in the crosslinking process and thus a decrease in the volume of the absorbed solvent. In addition, the incubation in PBS showed that the weight loss of the samples crosslinked after freeze-drying was lower than of the samples crosslinked before freeze-drying. It was 10% and 15% respectively for GelAlg1 after 3 days in PBS.

The influence of the amount of EDAC solution used in crosslinking was observed. A larger volume of EDAC increased the number of reactive groups and improved the effectiveness of crosslinking. With a small amount of the crosslinker, this process was insufficient and in consequence, the scaffolds' dissolution was observed after the first day of the incubation. This observation may explain the discrepancies in the literature regarding this topic [1,2]. This also indicates the need of further studies to determine the exact relationship between the gelatin degradation rate and the EDAC amount to ensure the process repeatability.

The FTIR analysis of the scaffolds before and after crosslinking showed differences in the relationship of the bands intensity. The bands from alginate and gelatin overlapped, however the increase in the intensity of the band at 1029-1080 cm^{-1} and 1405-1445 cm^{-1} was visible in relation to the amide bands at 1526 and 1630 cm^{-1}.

The second part of the study concerned crosslinking of gelatin fibres as they dissolved in the EDAC solution immediately after their immersion. The crosslinking process of the fibres was only possible after the NHS addition. The fibres after crosslinking showed stability in distilled water during the short-term incubation. The higher crosslinking efficiency in the EDAC+NHS solution results from the formation of the more stable amine-reactive intermediates [2]. Pure EDAC activates carboxyl groups and forms amine reactive O-acylisourea intermediate is unstable in water solutions, therefore it reacts with primary amines, and in consequence the amide bond formation is less effective. EDAC couples NHS to carboxyls, which are more stable than the O-acylisourea intermediate and the conjugation to primary amines is more effective.

Conclusions

The repeatability of the crosslinking process required a constant ratio of the hydrogel mass to the volume of the crosslinker. Changing the EDAC volume affected the effectiveness of crosslinking and thus the rate of the scaffolds degradation. The sequence of activities in the scaffolds production influenced their microstructure and stability. The crosslinking process did not cause premature rinsing of magnesium.

The addition of NHS to EDAC significantly improved the efficiency of crosslinking. The crosslinking of gelatin fibres was possible only in the EDAC+NHS solution.

Acknowledgments

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INHALABLE POLY(SEBACIC ANHYDRIDE) DELIVERY SYSTEMS OF AZITHROMYCIN AND CURCUMIN FOR THE TREATMENT OF RESPIRATORY INFECTIONS

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Introduction

Respiratory infections are a significant global health problem due to the fact that only a small amount of the drug administered orally or intravenously is able to reach the infected area in the lungs. Therefore, systemic therapies of high-dose antimicrobials are used and can lead to many side effects. Moreover, microbes gain resistance to antibiotics and are therefore harder to treat [1]. Chronic obstructive pulmonary disease (COPD) patients are at particular risk of recurrent lower respiratory tract infections [2]. Inhalable delivery systems in the form of biodegradable microparticles (MPs) can deliver the drug directly to the lungs and the encapsulation of the antibacterial quorum sensing inhibitor (curcumin) in them can reduce the minimum inhibitory concentrations (MIC) of azithromycin [3].
The aim of this study was to: i) obtain poly(sebacic anhydride) (PSA) MPs loaded with azithromycin (AZ) and curcumin (CU) of aerodynamic size between 1-5 μm; ii) analyze the encapsulation efficiency of CU; iii) evaluate the cytocompatibility of MPs in contact with human lung epithelial cells.

Materials and Methods

PSA was synthesized by melt condensation from sebacic acid (SBA) as described earlier [4]. 10 g SBA were refluxed in acetic anhydride (100 ml) under nitrogen for 40 min. The by-products of the reaction (excess acetic anhydride and acetic acid) were removed under vacuum. The remaining prepolymer was condensed at 150°C for 2 h under high vacuum (0.01 mm Hg) and nitrogen. The obtained polyanhydride was dissolved in methylene chloride, then precipitated in diethyl ether and petroleum ether in a volume ratio of 1:1, washed with petroleum ether and dried under vacuum. PSA was stored in the freezer. MPs were manufactured by a single emulsion method. AZ and CU were encapsulated by oil-in-water (o/w) emulsification. AZ or CU (AZ:PSA ratio or CU:PSA ratio of 1:10) were dissolved in 3 ml of the PSA solution in dichloromethane (DCM) (2% w/v) and homogenized with ultrasound (amplitude 40%) for 1 min. The obtained solution was poured into 20 ml of chilled poly(vinyl alcohol) (PVA) solution (8%) placed on a magnetic stirrer at 1500 rpm. The solvent was evaporated for 5 h and then the emulsion was centrifuged using 15000 rpm for 10 min. The supernatant was collected for encapsulation efficiency studies, and the centrifuged microparticles were freeze-dried. MPs with AZ alone and with CU alone were manufactured analogically. The morphology of the MPs was analyzed using optical microscopy and their size was measured using ImageJ software. Encapsulation efficiency of CU was evaluated using the fluorometric assay. The cytocompatibility of MPs was analyzed in contact with human lung epithelial cells BEAS-2B (ATCC, CRL-9609™) via AlamarBlue assay and live/dead fluorescent staining. Cells were seeded in the 96-well plates (10,000 cells/well) and incubated (37°C; 5% CO_2) for 24 h. Then, medium was replaced with MPs suspensions in Dulbeco modified Eagle's medium (DMEM) up to 1000 μg/ml and cultured for another 24 h.

Results and Discussion

MPs with a size suitable for inhalation (1-5 μm) were manufactured successfully at an efficiency of 88.5%. Particles were homogeneous (diameter range 0.46-6.74 μm), round, regular shape and of yellow color. The encapsulation efficiency of CU in MPs containing AZ was 43.4 ± 1.4%, while the encapsulation efficiency of CU in MPs without AZ was 44.7 ± 1.3%. Results confirmed the lack of impact of AZ on CU encapsulation efficiency. Obtained MPs showed no cytotoxic effect against the BEAS-2B cells at concentrations up to 100 μg/ml. Moreover, individual living cells were observed in the images obtained during live/dead fluorescent staining even for a MPs concentration of 1000 μg/ml. This allows us to conclude that curcumin has a positive effect on cell viability.

Conclusions

To sum up, PSA particles loaded with AZ and CU are promising inhalable delivery systems for the treatment of respiratory infections especially for COPD patients. However, further studies such as evaluating release and degradation kinetics and examining the synergism of AZ with CU against bacterial strains should be conducted.

Acknowledgments

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

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NANOCOMPOSITE COATINGS WITH CONTROLLED SURFACE PARAMETERS

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Introduction

Due to the dynamic development of therapeutic and diagnostic methods, modern medicine faces many new challenges. One of them is the need for new, highly functional materials, thanks to which new, more effective solutions for medical therapy and diagnostics will be developed. The requirements for modern medical materials have become very complex, which results, among others, from the rapidly increasing pollution of the environment, as well as the difficulties posed by the world of microorganisms that reach the human body in an uncontrolled manner. Increasingly, there is a need for materials with high durability, the use of which can significantly reduce the amount of medical waste, and materials with controlled surface properties. For medical applications, materials with antibacterial properties are needed, those that can be colonized by the cells of a living organism, and also those to the surface of which cells do not adhere. Materials with antibacterial properties, with electrical conductivity adapted to the appropriate cellular reaction include, among others one of the solutions for the development of a new generation of bioelectrodes, biosensors and systems stimulating tissue regeneration. The subject of our research was a group of nanocomposite coatings on the metal surface, consisting of carbon nanoforms and organosilicon polymer, obtained by EPD electrodeposition. Such systems are characterized by high durability, suitable electric conductivity and exhibit antibacterial properties [1]. The specific goal of our research was to show how, using appropriate process conditions and using the electrodeposition method, one can control the properties of the surface of selected nanocomposite materials, important from the point of view of medical applications

Materials and Methods

Nanocomposite coatings were formed on the titanium surface, using a siloxane sol and carbon nanotubes (CNT-NanoAmor, USA) by means of the EPD method. Additionally, the coatings were prepared from CNT alone and pure polymer. Sol solution was prepared by the catalytic hydrolysis of the siloxane precursors, i.e. methyltrimethoxysilane (MTES, ≥98%, Sigma-Aldrich, USA) and dimethyldiethoxysilane (DMDES, ≥97%, Sigma-Aldrich, USA). The coatings were obtained by a co-deposition process (siloxane sol containing dispersed CNTs) and by a sequential process. In a sequential process, the titanium surface was first coated with the CNTs and then with the siloxane salt. The nano and microtopography of the obtained coatings were determined, their surface energy and its components were examined. In biological studies, mouse fibroblasts from the L929 line (European Collection of Cell Cultures, Sailsbury, UK) were used, grown in DMEM (Dulbecco's Modified Eagle Medium). The studies included the assessment of the metabolic activity of the cells (resazurin reduction test, AlamarBlue) and the viability and morphology of the cells (live / dead fluorescence staining).

Results and Discussion

The results of the study on nanocomposite coatings modified with carbon nanoforms show that, depending on the method used, the micro and nano-surface topography may vary in a wide range. Depending on the deposition time of both components, the sequential deposition coatings have a smooth surface or one whose morphology is determined by the presence of CNTs. FIG. 1A. shows the SEM image of the coating obtained in the sequential deposition process; it shows densely arranged nanotubes, covered with a thin layer of siloxane. On the other hand, the coatings made in the co-deposition process, in the microscopic image, are also characterized by a different morphology (FIG. 1B). Both nanometric areas are visible, which are caused by agglomerates of carbon nanotubes and those related to the presence of pure polymer. In the co-deposition method, besides the factors mentioned above, the surface image depends to a large extent on the properties characterizing the population of nanotubes that make up the suspension used in the EPD process, i.e. factors important from the point of view of the dispersion of the nanoadditive in the siloxane sol. In vitro studies in contact with cells indicate that the surface created by a dense system of carbon nanotubes, covered with a thin polymer coating, promotes the adhesion of fibroblasts (FIG. 2A). Moreover, on the surface of the coating obtained in the process of co-deposition, the different morphology causes that only some areas of this sample are inhabited by cells (FIG. 2B) Cells adhere to those sites of the nanocomposite coating that are characterized by nanotopography induced by the CNTs present on the sample surface. On the other hand, surfaces with a smooth topography, where the nano-additive only affects the surface chemistry, are areas to which cells do not adhere.

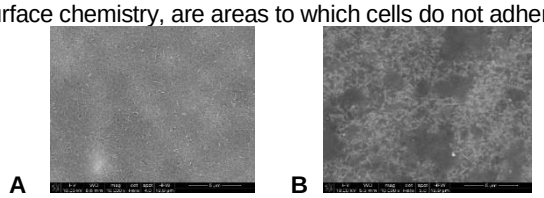

FIG. 1. SEM images; sequentially deposited layer (A) co-deposited layer (B).

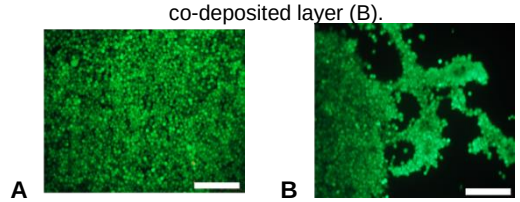

FIG. 2. Live/Dead assay of fibroblast in contact with; surface of sequentially (A) and co-deposited (B) layers.

The topography of coatings manufactured in the process of electrophoretic deposition depends both on the method (co-deposition, sequential deposition), process variables (time, voltage) and the amount and chemical structure of the carbon modifier surface.

Conclusion

The results of our research indicate that nanocomposite coatings made of organosilicon polymer and carbon nanoforms are materials with high potential for applications in medicine, as well as broadly understood environmental protection.

Acknowledgments

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ANTIMICROBIAL AND BIOACTIVE PROPERTIES OF Si/Ti LAYERS MANUFACTURED BY SOL-GEL METHOD

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Introduction

Research on increasing of the functionality of layers applied onto implant surfaces has been an issue addressed for years. This is primarily related to the development of layers with utilitarian properties both in terms of biology and material, but also in terms of usability (ease of application to different geometries and forms of implants). The primary task of the layers in this context is: to protect the surface of the implant from destructive activities (e.g., from corrosion), but also the additional role of the layer as an intermedium, i.e., an intermediate layer between the actual material and the external environment. This has a particularly important impact in the first stage of the cellular response. Inhibiting the bacterial biofilm and stimulating processes related to adhesion, proliferation and, above all, to the chemical bonding of the implant to the tissue is the task that modern layers face [1].

The aim of the study was to select a ratio of polysiloxane precursor and titanium precursor that would enable to achieve a continuous film characterized by reduction of bacterial biofilm against two key bacterial strains: Gramm negative (*E. coli*) and Gramm positive (*S. aureus*).

Our studies indicate that polisiloxasium-titanium layers obtained by this method have antibacterial features with simultaneous bioactive potential. Their additional advantage is the possibility of applying the mentioned layers to finished products (implants) based on metallic, ceramic, glass, as well as polymeric and carbon materials, regardless of the form of the material (solid/porous).

Materials and Methods

Commercial chemical reagents of analytical grade purity were used to prepare the sols. As an organic precursor for the preparation of titanium and polysiloxane-titanium solvents were 98% $Ti(OC_3H_7)_4$ solution (Sigma-Aldrich), 98% TEOS - $Si(OC_2H_5)_4$ solution (Sigma-Aldrich), 99.8% C_2H_5OH ethyl alcohol solution (Avantor SA) was used as a solvent. The catalyst for the polycondensation reaction was 99.8% acetic acid solution CH_3COOH (Avantor SA). Layers were applied by infiltration or dip coating. The following were used as substrates: steel plates and glass substrates. Microbiological reagents including: PCA agar, NB nutrient medium, SCDLP release agent (Biomaxima SA) were used. Salts necessary for buffer preparation including $CaCl_2$, NaCl, NaH_2PO_4, were from Avantor SA.

To study the quality of the layer, the following were used: microscopic observations using a scanning electron microscope SEM (Nova NanoSEM, FEI), the elemental composition of the layer was studied using the EDS method. The antibacterial properties of the films applied to substrates were examined using the guidelines of the PN-EN ISO 22196:2011 standard for determining antibacterial properties on plastic materials and other non-porous surfaces. Two commercial strains of reference bacteria were used: *S. aureus* ATCC 6538P and *E. coli* ATCC 8739. Bioactivity testing of the films was carried out in simulated SBF plasma prepared according to the recipe developed by Kokubo et.al (2004); incubating the film-applied materials for 7 days and observing the film surfaces using a scanning microscope (Nova NanoSEM, FEI). The physicochemical properties of the layers (wettability/surface energy) were tested by the sitting drop method using a Krüss DSA25E goniometer.

Results and Discussion

The sol-gel technique is an easy and effective way to produce solid layers on both glass and metallic substrates based on both titanium and polysiloxane-titanium sol. The presence of polysiloxane sol is responsible for obtaining a solid layer. The greater the addition of titanium sol, the more cracked the layer is, as evidenced by SEM microscopic observations. EDS analysis showed that polysiloxane-titanium coatings are characterized by heterogeneous distribution of silicon and titanium. The amount of titanium in the layers does not exceed 10% by weight. The studied layers differ in surface energy and wettability. Their value is closely related to the presence of Ti-based sol: lower surface energy and higher wettability angle compared to the control sample (glass/steel substrate) are exhibited by layers with more titanium. The values of the polar component of the surface free energy recorded for the tested layers with titanium is lower by 15 mN/m, which, according to the literature, is the limit for promoting bacterial cell adhesion [2].

The coatings obtained on the steel substrate exhibited bacteriostatic properties, as evidenced by both the obtained value of the reduction factor and the bacterial morphology observed on the substrate coated with the tested layer (FIG. 1). The titanium layer had higher antibacterial activity against Gramm negative bacteria, while for the polisiloxane-titanium layer it was higher for Gramm positive bacteria. Interestingly, for layers coated with layers above 500 µm, bacteriostatic properties were noted against both E. coli and S. aureus. This leads us to suppose that the thicker the layer or its different structure in the boundary layer formed during the polycondensation process, the more the chemical groups responsible for inactivating the bacterial cell wall are exposed to the surface.

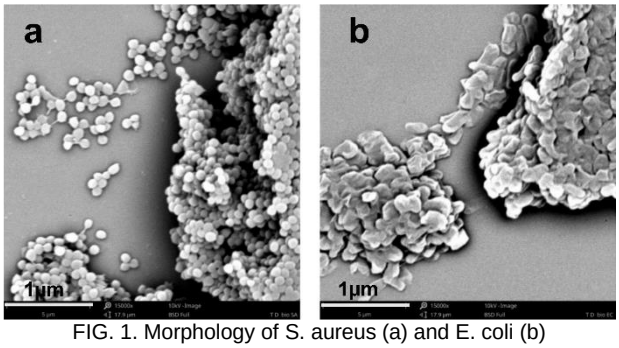

FIG. 1. Morphology of S. aureus (a) and E. coli (b) bacterial cells on Si-Ti layer.

Conclusions

Layers based on polysiloxane-titanium sol as well as titanium sol show bacteriostatic properties and can be applied to surfaces of varying nature. An important factor here seems to be the thickness of the layer and this requires further research

Acknowledgments

Research project supported by program IDUB "Excellence initiative – research university" for the AGH University of Science and Technology". Project ID 4220.

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CARBON-CARBON COMPOSITES IN A PYROLYTIC CARBON MATRIX – OPTIMIZATION OF THE PRODUCTION PROCESS AND PRELIMINARY ASSESSMENT OF THEIR SELECTED PROPERTIES

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Introduction

The nervous system performs one of the most important functions because it allows to control the functioning of the entire body. One of the many disorders of the nervous system are neurodegenerative diseases, leading to damage to nerve cells in the brain area. These diseases are characterized by gradual and slow development. Treatment of neurodegenerative diseases is mainly based on drug therapy, although deep brain stimulation (DBS) can also be used. DBS is based on the implantation of electrodes into the patient's brain, the task of which is to stimulate nerve cells, restoring the appropriate patterns of neural signalling. Currently used electrodes are made of metals such as stainless steel, platinum and their alloys, e.g. platinum-iridium [1]. Metal electrodes, despite meeting the therapeutic assumptions, have disadvantages that will reduce the effectiveness of the therapy. Therefore, the research is focused on finding an alternative material for the production of electrodes. Carbon materials are potential candidates, mainly due to their physicochemical and biocompatibility properties [2].
The aim of this work is to optimize the process conditions of the production of carbon-carbon composites (C-C composite) based on carbon fibers and two types of carbon matrices, i.e. phenol-formaldehyde resin pyrolysate and pyrolytic carbon (PyC) obtained by chemical vapor deposition (CVD). The obtained composites are to serve as electrodes used to stimulate the cells of the central nervous system. The geometry of these electrodes are cylindrical rods with a diameter of less than 1 mm.

Materials and Methods

In this work low-modulus carbon fibers from the polyacrylonitrile (PAN) precursor (Sigrafil, SGL Carbon company) were used. A phenol-formaldehyde resin (DP-02, from Lerg) was used to produce the core of the carbon-carbon composites. The composites based on carbon fibers and resin were obtained in the form of rods. The next step was the carbonization of these samples at 1000°C with 7°C/min progress and 30l/h nitrogen flow. The next step was to obtain coatings of pyrolitic carbon, use the resistance heating system and the CVD method for this purpose. Methane was used as the carbon source gas in the CVD method. The conditions of this process were optimized and constituted the main goal of the conducted research. The obtained samples were examined by means of optical microscopy, SEM, XRD and Raman spectroscopy. The results of the electrical tests were carried out using the four-point method. Initial *in vitro* biological studies were also performed on the SH-SY5Y human neuroblastoma cell line.

Results and Discussion

Optimization of the process of both the preparation of the C-C composite core and the PyC coating led to the production of composites in the form of rods with a diameter between 100 and 200 µm and a length of 3 cm. Thanks to the optimization of the CVD process including synthesis time and the amount of carbon source gas, it was possible to obtain composites with PyC coatings of various thicknesses, i.e. from several dozen nanometers to several dozen micrometers. The microstructure and hierarchical structure of the obtained composites was presented in the SEM micrograph (FIG. 1).

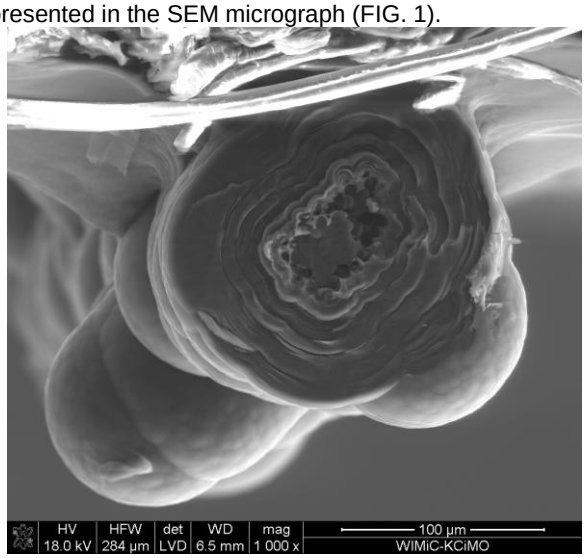

FIG. 1. SEM microphotograph of the cross-section of C-C composites.

From the structural point of view, carbon-carbon-composites with PyC coatings were characterized by a greater structural order compared to composites based on phenol-formaldehyde resin and after its carbonization, as evidenced by the results of XRD studies and Raman spectroscopy. The results of electrical tests of C-C composites with PyC coatings showed a twofold increase in electrical conductivity in relation to the carbon composite without PyC (form about 350 S/cm to 690 S/cm). Initial toxicity studies performed using the LDH test showed no toxic effects of C-C composites with a PyC coatings on SH-SY5Y cells. Cell viability in contact with C-C composites after 48 h of culture was higher than for the control sample (bottom of the culture well) and higher than for Pt wire, which is often used as electrode material for deep brain stimulation.

Conclusions

The resistance heating method combined with the CVD method is very effective in obtaining and controlling the thickness of the PyC coating and obtaining carbon-carbon composites in the form of rods with a diameter of 100-200 µm. Preliminary results of structural, microstructural and electrical tests of the obtained composites indicate the influence of the PyC coating, while the preliminary results of *in vitro* biological tests show the positive effect of PyC on the cellular response.

Acknowledgments

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EVALUATION OF COMMERCIALLY AVAILABLE NiTi WIRES FOR CARDIAC SURGERY

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Introduction

Only NiTi alloys have found practical applications for implants and medical instruments from the family of alloys showing the shape memory effect. It is due to the relatively good corrosion resistance and tolerance in a biological environment [1].
Their usefulness was assessed based on the existing shape memory effect. There are three effects: superelastic phenomena, one and two-way shape memory effect [2].
For medical applications, the superelastic effect is used the most often. It consists in changing the shape under the influence of external load. After unloading, the material returns to its original shape. In the case of NiTi wires, this effect, e.g., during stretching, increases the length even up to 10%.
The present work provides a brief overview of the preliminary evaluation and verification of commercially available Ni-Ti wires for braided cardiac implants.

Materials and Methods

Commercially available Ni-Ti wire with a diameter varied from 0.1 mm up to 0.75 mm was used to evaluate their suitability for cardiac implants use through occurring reversible martensitic transformation.
The martensitic transformation's presence, reversibility, and stability were assessed based on the measured thermograms. Measurements were performed on a Metler Toledo DSC-1 differential scanning calorimeter (DSC). Measurements were performed from -120ºC to 120ºC at a rate of 10 deg./ min under a protective argon atmosphere.

Results and Discussion

FIG. 1 shows an example of the thermograms measured for 0.15 mm wire. In order to confirm the repeatability of the martensitic transformation, two thermal cycles were measured. First, the obtained results proved the reversible nature of the martensitic transformation. It meets the criteria for the shape memory effect occurrence. It was proved by the course of the cooling/heating curves. It means: if there was a thermal peak or peaks on the heating curve - their equivalents appeared on the cooling curves, respectively.
The characteristic temperatures of the martensitic transformation (M_s, M_f, A_s, A_f), were determined from the measured thermograms. For medical applications the most important is the end temperature of the reverse martensitic transformation - A_f. The results, as a function of wire diameter, are shown in FIG. 2.

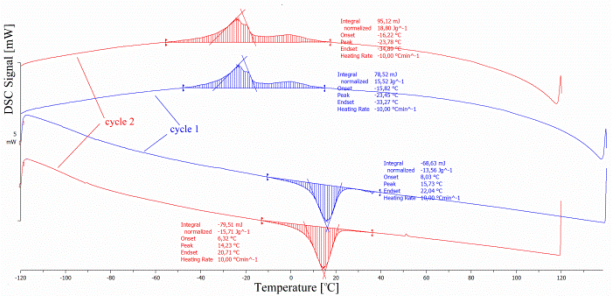

FIG. 1. DSC cooling/heating curves measured for wire with diameter of 0.15 mm.

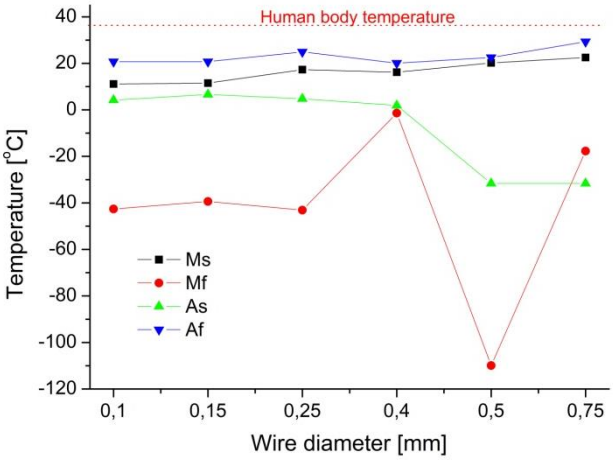

FIG. 2. Characteristic temperatures of the martensitic transformation determined from DSC measurements.

Wires with 0.10, 0.15, and 0.25 mm diameters presented similar characteristics. In these cases, the reverse martensitic transformation was additionally characterized by the highest transformation enthalpy. Such behavior is favorable when their medical application is considered. For the wire with 0.5 mm diameter there was observed a slight increase in the A_f temperature up to 22.5ºC. These wires revealed the most excellent reserve for the safe use in the case of its application for a human cardiac implants. For the 0.75 mm wire, the A_f value reached almost 30ºC, what adversely reduced the reserve for its safe use (36.6ºC).

Conclusions

Irrespective of the diameter, the presence of a reversible martensitic transformation was confirmed in all studied wires. Moreover, the martensitic transformation occurs below the human body's temperature. It guarantees the occurrence of superelasticity and its application in medicine and veterinary medicine.

Acknowledgments

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THE EFFECT OF BIOACTIVE CERAMIC MODIFICATION ON MECHANICAL PROPERTIES OF POLYURETHANESACCHARIDES

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Introduction

The rapid development of biomaterials for applications in implantology, cell culture or artificial organs has been observed in recent years. This is linked to the increasing number of procedures for bone diseases treatment directly influenced by current lifestyle.

Two groups of bone cements are most commonly used in implantology: polymer-based poly(methyl methacrylate) (PMMA) and ceramic-based calcium phosphate cements (CPC) [1,2]. Unfortunately, none of the above groups of materials is without any disadvantages, so further research is being conducted on further development of currently available materials, as well as on the use of new materials as bone cements. Hence, the aim of this study was to determine the effect of hydroxyapatite (HAp) on the mechanical properties of polymeric bone cements based on polyurethanesaccharides [3-5].

Materials and Methods

Poly(ethylene oxide) – PEG 2000 as soft segment, polysaccharides (alginate or starch) as crosslinkers and 1,4-butanediol (BDO) as the chain extender were used to obtain polyurethane foams. Hydroxyapatite at 2.5; 5; 7.5 and 10 wt% load was used as a bioactive modifier.

In the first step, poly(ethylene oxide) was dried and melted. This process was carried out at 45-50°C in an inert atmosphere (nitrogen). A catalyst and 1,6-hexamethyl diisocyanate (HDI) were then added. The mixture was heated at approximately 55°C for 40 minutes. Polysaccharide, hydroxyapatite powder and BDO were added to the reaction mixture. The crosslinking/chain extension process was carried out in a heating oven in two stages: (1) 30 minutes at 80°C, (2) 2 hours at 60°C. From the material thus prepared, samples were cut for tests according to the ISO standards.

Scanning electron microscope (SEM) investigations, mechanical properties (compressive strength) tests, dynamic mechanical analysis (DMA) and ultrasonic testing were carried out.

Results and Discussion

Microstructure studies show that the addition of alginate leads to the higher porosity of the material than the addition of starch. The pores are more spherical creating an open porosity throughout. Regardless of the polysaccharide addition, an increase in the hydroxyapatite content increases the porosity of the composite. In contrast, the hydroxyapatite itself is heterogeneously distributed. This is the result of agglomeration of the HAp powder with the liquid prepolymer.

The addition of bioactive ceramics yields different properties depending on the polysaccharide used. Higher Young's modulus values were obtained when starch was used, with the highest values obtained for the addition of 7.5 wt.% and 10 wt.% of HAp, with E values of 26.6 and 26.3 MPa, respectively. In contrast, the highest Young's modulus values for alginate were obtained for contents of 2.5 and 5 wt.% HAp - 26 MPa and 17MPa, respectively.

Ultrasonic tests show very similar elastic modulus dependencies on the amount of HAp additive to those obtained from mechanical tests.

Dynamic mechanical tests allowed to determine the range of glass transition temperatures, as well as the viscoelastic properties of the foams. The glass transition temperatures for all samples are similar and are from ca. -50°C +/- 5°C to -37°C +/- 3°C. However, no significant correlation could be found between the amount of HAp added to the foam and the glass transition temperature shift.

The storage modulus determined during the test corresponds to the elastic properties of the material. The obtained results indicate that the elastic properties of polyurethane foams decrease to a very large extent with an increase of temperature. In addition, the elastic properties weaken most when the glass transition temperature is exceeded. At ambient temperatures (20-25°C), the best elastic properties among the alginate composites were recorded for those with 7.5 wt.% and 10 wt.% HAp - 23.5-32.5 MPa and 27.3-37.1 MPa, respectively. These values decrease with temperature, and the materials containing 7.5 wt.% HAp show greater stability. In the case of starch-modified materials, only the addition of 2.5 wt.% HAp resulted in a slight improvement in properties compared to the reference sample, while already at ~37°C the reference sample, i.e. without HAp, showed the best performance.

Conclusions

The use of alginate as a crosslinker leads to formation of highly porous materials with open porosity throughout, but with poor mechanical properties.

The use of starch as a crosslinker makes it possible to obtain material with a much higher Young's modulus.

For foamed materials, foams with 2.5 and 5 wt.% HAp from the alginate group, and with 7.5 and 10 wt.% HAp for starch-based composites have the best physico-chemical properties.

Because of the mechanical properties, the fabricated materials can be considered as scaffolds, e.g. for cell culture and/or in implant applications that do not require the transfer of high mechanical loads.

Acknowledgments

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FATIGUE TEST OF METAL WIRES FOR A THERAPEUTIC DEVICE FOR CLOSING THE VENTRICULAR SEPTAL RUPTURE

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Introduction

Perforation of the heart is a severe condition that can occur as a result of a medical disorder, as a congenital defect or trauma from an accident. There are many research conducted to develop a therapeutic device dedicated to such conditions but still very few treatments for such a phenomenon exist on the market. A novel implant need to fulfil very strict requirements. Both, material and it's mechanical properties along with a special design need to be carefully selected and matched for this purpose. One of the most important factor for cardiac implants is their fatigue resistance. This work was dedicated to mechanical characterisation of materials that can be used for such a purpose. They were subjected to uniaxial tensile tests and fatigue tests to determine their strength parameters. The work aims to verify which of the wires meet the requirements and could be possibly used for a prototype device preparation.

Materials and Methods

For the laboratory tests stainless spring steel St302 wires (with diameter: 0.15 mm; 0,2 mm; 0,3 mm; 0,4 mm; 0,5 mm; BHH Mikrohuta, PL) and NiTi wires (with a diameter: 0,25 mm; 0,5 mm; 0,75 mm; Kellogg's Research Lab, New Boston, NH, USA) were used. Static mechanical tests including uniaxial tension and wire bending were carried out on a Zwick/Roell 1435 universal testing machine. Fatigue tests were carried out on Electrodynamic Testing Machine STEPLab EA-UT-05 and were based on previously performed static tensile tests. The test itself used the low-cycle method.

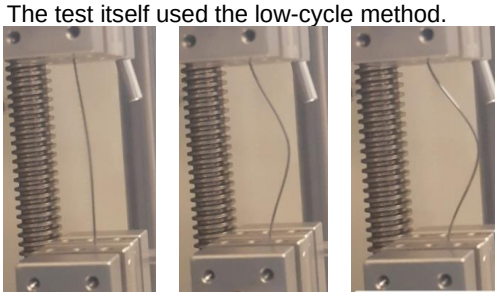

FIG. 1. Fatigue "step by step".

Results and Discussion

The effect of fatigue is evident for wires with smaller diameters of 0.15; 0.2 and 0.3, for which the tensile strength, maximum stress in the elastic range (proportional limit), strain at failure and Young's modulus decreased. For wires with diameters of 0.4 and 0.5, the mechanical properties remained the same. In the case of steel wires, it is therefore difficult to say unequivocally whether they show fatigue resistance in the investigated load/strain range. It seems to depend on the diameter of the wires. In addition, the stresses generated in the initial and final phases of the test (first and last cycles) are the same, i.e. the nature of the fatigue curve does not change.

For nitinol wires, no significant changes in properties were observed after cyclic testing. Pseudo-elasticity and a wide range of reversible deformations were retained. Their tensile strength, proportional limit and Young's modulus remained unchanged. Due to the mechanical characteristics, it can be concluded that nitinol in wire form can be successfully used for the construction of a therapeutic device. Despite the testing of nitinol at different test points comparable results were obtained.

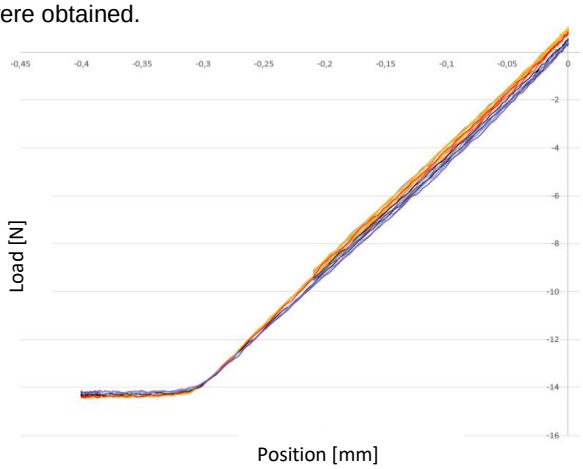

FIG. 2. Example of force-displacement relationship for 0.25 mm diameter nitinol wire.

Discussion and conclusions

Medical steel wires with smaller diameters, i.e. 0.15 to 0.3 mm, become weaker as a result of fatigue. Their mechanical properties decrease as the number of fatigue cycles increases, with the maximum stresses at the beginning and end of the cyclic tests being the same (the curves in the graph overlap). Despite the weakening of the material, steel wires could serve in the construction of a prototype therapeutic device if their strength or modulus were considered Young's modulus. However, a small deformation in the elastic range could be a limitation in using steel for such a device. There is a risk of plastic/plastic deformation during operation which is unacceptable for such an application.

Nitinol wires retain their mechanical properties despite prolonged operation. They do not lose their pseudo-elastic character and a wide range of reversible deformations. Unlike steel, the Young's modulus value of nitinol and its strength did not decrease with service time. Analysing the mechanical characteristics of nitinol wires and their resistance to fatigue, it can be concluded that they will be able to successfully serve in the construction of the prototype of the designed therapeutic device.

Acknowledgments

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ELASTIC BEHAVIOR OF LASER-BEAM-WELDED STAINLESS STEEL TAPES FOR CARDIOVASCULAR APPLICATIONS

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Introduction

Despite advances with new generation stents, there remains some atypical coronary anatomy where optimal stenting continues to be a challenge. Laparoscopic operational procedure of vascular/coronary stent implantation is a golden standard in such cases. Firstly implant is compressed and placed inside a long hollow tube (catheter) inserted from the wrist or the groin and guided (using X-rays) all the way to the narrowed artery. Then it is precisely positioned and expanded either with the inflated balloon (balloon-expanded) or by spring-back effect (self-expanded) to fit the stent to the artery wall. This is so called one-way shape change. Stents are usually made from metal such as stainless steel, platinum-chromium or cobalt-chromium and NiTi alloys. Novel approach assumes that the implant should not be a stiff cage widening the lumen of the artery but should follow and fit to its shape changes during cardiac cycle not causing any harm. For such purpose braided self-expandable stents made of very thin wires are used. However, their elastic spring-back force is rather low and the stent may not be properly fixed. The idea is to use - instead of the inflatable balloon - additional elastic internal supporting structure which helps the stent to expand. After the stent is positioned and properly expanded the supporting structure is removed. This research is focused on verifying characteristic of an elastic component of such a supporting structure made of rigid stainless steel.

Materials and Methods

Stainless steel AISI302 tape 1,0 x 0,2 mm and round wire ø = 0,3 mm (BHH Mikrohuta, PL) were used. Samples were firstly formed and then welded to a holder (Jewelry Mini Spot Laser Welder JCW-100; PixoLaser, China). Welding parameters: Curr = 40%, Freq = 6,0Hz, Puls = 0,8 ms, Spot = 0,1 mm. Length of the arm: 40 mm, bending angle = 35°, length of the weld seam = 5 mm. To obtain different stiffness three types of arm construction were designed: a) steel tape only; b) steel tape stiffened with a welded round wire on the surface in half of the arm length; c) steel tape extended in half of the arm length with round wire (FIG. 1).

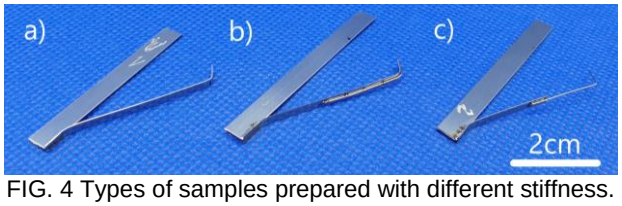

FIG. 4 Types of samples prepared with different stiffness. a) steel tape only; b) steel tape stiffened with a welded round wire on the surface; c) steel tape extended with round wire.

Elastic behaviour of the samples was characterised by deformation force vs. displacement. Mechanical testing in single-point-bending test was conducted on Zwick Roell Retro 1435 universal testing machine. Normal conditions, piston speed 2 mm/min. Deformation force was recorded and the test was stopped when displacement of piston achieved 10 mm (FIG. 2).

FIG. 2. One-point-bending test. a) start; b) finish.

Results and Discussion

After all types of samples were tested it occurred that regardless to the construction of an arm the deformation force was comparable in all cases. The stress-strain curves were also very similar. It indicates that the stiffness of the stainless steel tape itself is crucial and deformation is localized only in curved and welded areas, thus to change stiffness of the arm different geometry of the tape should be applied. Furthermore, it can be stated that laser welding did not effect the characteristics of the material. Although laser beam causes material melting, the size of the spot is very small (0,1 mm) and the heating zone is also very limited. Probably, the portion of the energy is insufficient to cause any structural changes in wider range.

Conclusions

AISI302 stainless steel can be used for a self-expandable stent additional support device. To adjust and tailor its stiffness and elastic characteristics various tape geometry should be used. No other construction variations eg. stiffening with welded wires on the surface are need. Deformation zone is localized only in the narrow area, close to the curvature and weld seam.
It was proved that laser welding is appropriate method for implant fabrication. To verify structural changes after welding process additional detailed research with SEM, EDS, TEM is required.

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