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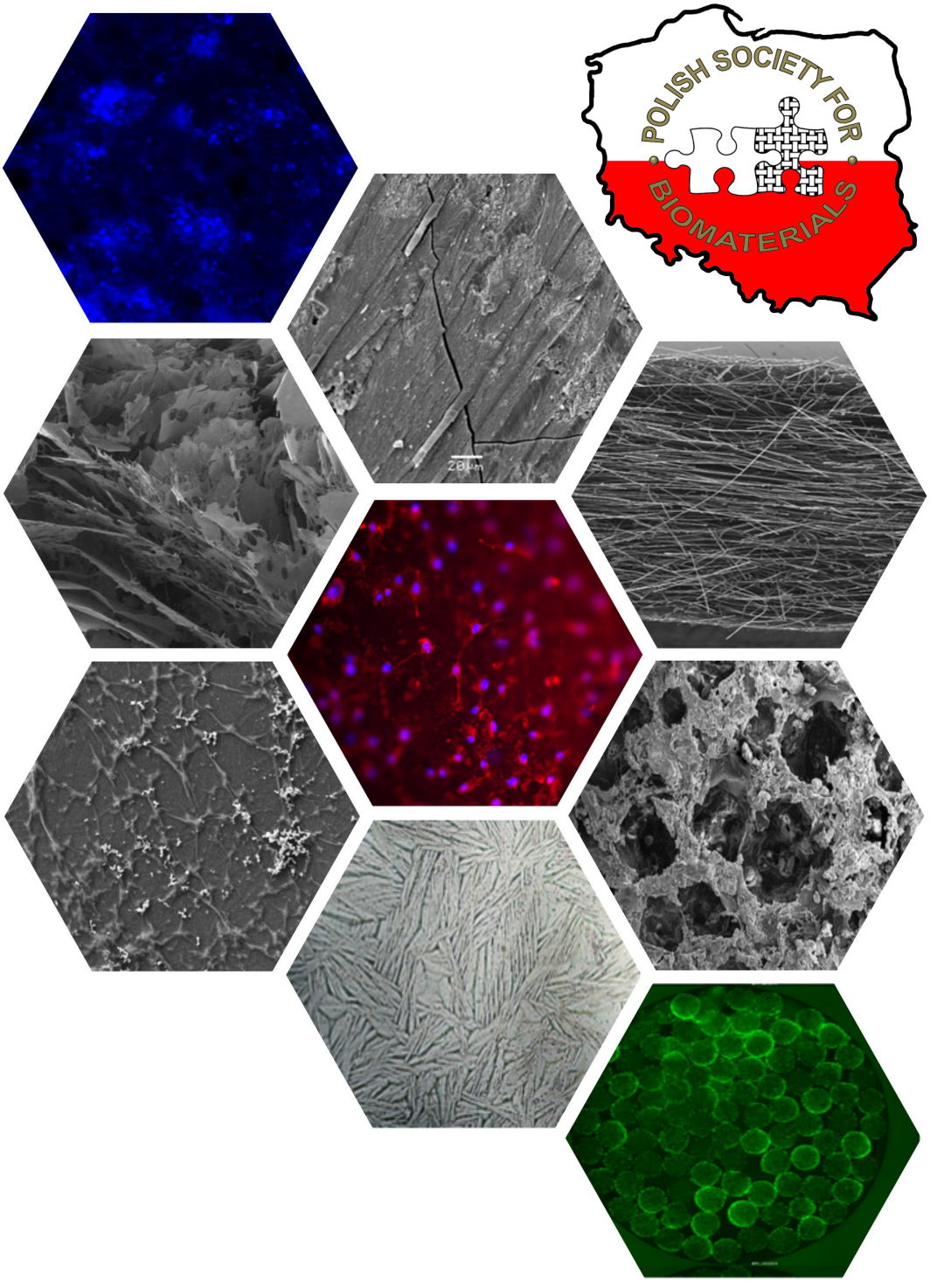

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2018/2019

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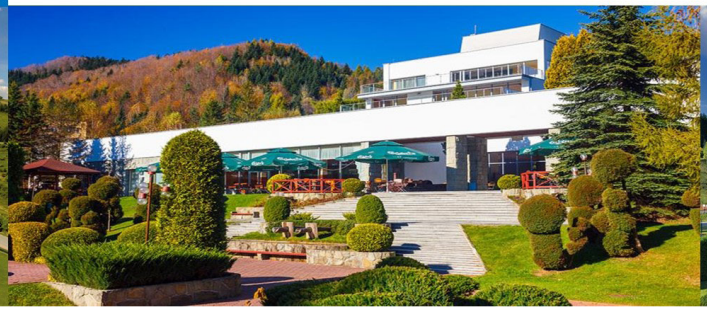

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ARGETING NEURONS OVERCOMING EXTRA AND INTRACELLULAR BARRIERS WITH BIOMATERIAL-BASED VECTORS TO PROMOTE NEUROPROTECTION AND NEUROREGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 7]

Nervous system problems are common and encompass a large spectrum of traumatic injuries, diseases or iatrogenic lesions. The poor regenerative capacity, particularly in the case of the central nervous system (CNS), cannot be attributed to an intrinsic inability of neurons to sprout and re-grow after injury, as axons are able to regenerate in the presence of a permissive growth environment. One of the challenges facing the neuroscience field is the development of effective therapies that can enhance the regenerative capacity of the nervous system based on the advances achieved in basic research.
We have been dedicated to using nano-enabled solutions to the design of new therapeutic approaches towards the enhancement of the process of nerve regeneration. In this talk, particular emphasis will be given to the design of biomaterial-based nanoparticles for targeted nucleic acid delivery to neurons to promote neuroprotection and neuroregeneration.

Two biomaterial-based vectors will be discussed:
- polymeric nanoparticles based on thiolated trimethyl chitosan to mediate targeted gene delivery to peripheral neurons upon a peripheral and minimally invasive intramuscular administration [1];
- dendrimer based vectors [2] for brain delivery in the aftermath of stroke.

Emphasis will be given to the application of novel strategies proposed to assess the potential of the developed systems and contribute to the design of more efficient nucleic acid delivery vectors. Namely, the use of:
- imaging flow cytometry - a high throughput technique with unique features that combines the statistical strength of flow cytometry with image acquisition of every event - to unravel some critical aspects for vector formulation [3];
- atomic force microscopy as a tool to assess the specificity of targeted nanoparticles in biological models of high complexity [4];
- microfluidic-based platforms to mimic the in vivo administration of neurotropic nanoparticles [5].

Acknowledgments
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NATURALLY-DERIVED HYDROGELS AND NANOCOMPOSITES AS BUILDING BLOCKS OF SCAFFOLDS FOR TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 8]

Introduction
Naturally-derived hydrogels are developed as bioinspired polymer-based biomaterials for the fabrication of scaffolds and as key ingredients for the engineering of biomaterial surfaces for controlled biointeractions. Such hydrogels can be produced by cross-linking of pure polymers [1,2] or cross-linking of natural polymers derivatives [3,4], as multicomponent systems with combined properties [3,4], they can be (bio)functionalized for specific applications [5] or used as components in (nano)(bio)composites [6,7]. Recently, hydrogel precursors of natural origin have become appealing for development of viscous formulations for (bio)fabrication such as electrospinning and 3D printing [6,7].

Materials and Methods
We developed naturally-derived hydrogels based on pure or modified proteins and polysaccharides, available for tissue regeneration applications. Pure or modified gelatins, mucin, sericin, alginate, nanocellulose and pectin are used to tune the characteristics of different scaffolds. Obtained through covalent or ionic cross-linking, through network-forming photopolymerization sometimes combined with radical polymerization of synthetic monomers, biofunctionalized with cell-adhesive peptides, our hydrogels are designed to tissue regeneration and generation of bioartificial implants.

Results and Discussion
The design, synthesis and fabrication of the scaffolds based on naturally-derived hydrogels are based on bio-inspired approaches. The surface and bulk microstructure and functional properties including elasticity, permeability, biodegradation of the developed scaffolds are evaluated by advanced investigation methods since they play important roles in the stimulation of suitable biointeractions with cells [1-7]. We established methods for the use of hydrogels in the fabrication of porous scaffolds, membranes, films, fibers or particles with predefined properties. Conventional fabrication techniques and more recently electrospinning, electrostatic bead generation and 3D printing are used to produce cell-interactive scaffolds for tissue regeneration. Rheological properties of precursors, cell-friendly cross-linking protocols, specific biofunctionalization and stability tests are required for each type of hydrogel and application. Special consideration is given to the behaviour of hydrogel-based scaffolds in physiologically simulated conditions including bio-dynamic testing in simulated media, cell-interactions in 3D systems including the use of cell spheroids. As an example, FIG. 1 presents nanobiocomposite particles with controlled microstructure and composition designed to obtain osteoblasts adhesive surfaces, fabricated by electrostatic bead generation. FIG. 2 is representative for the stimulation of cell adherence by coating of a polypropylene mesh for abdominal wall fixation with methacrylamide gelatin hydrogels.

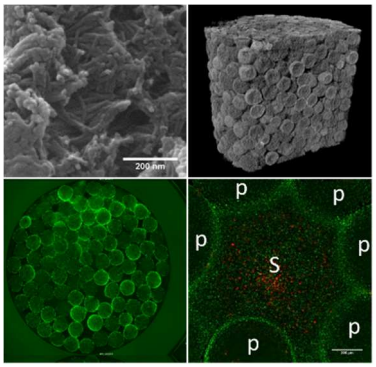

FIG. 1. Bio-inspired nanobiocomposites based on naturally derived hydrogels and nanoapatite. a, b - morpho-structural features (a – SEM; b – microCT); c – osteoblasts adhere on the surface of the beads ; d – the osteoblasts from a spheroid (S) immediately covered the surface of nanobiocomposite particles (P) proving the potential of such scaffolds both as a bone-filling biomaterial but also as a 3D scaffold for the next generation of smart bone regenerative fillers seeded with autologous cells, for personalized medicine.

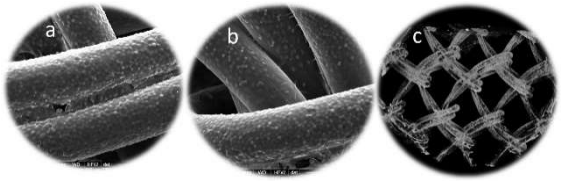

FIG. 2. Polypropylene meshes coated with gelatin-based hydrogels promote fibroblasts adhesion: a,b – SEM micrographs, c – micro-CT

Conclusions
Our research on the development of naturally-derived hydrogels for tissue regeneration emphasizes the importance of microstructural control at the interface biomaterial-cells and acknowledge the need for more appropriate *in vitro* testing conditions to simulate cellular phenomena required for tissue regeneration.

Acknowledgments
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THE EFFECT OF PLASMA TREATMENT ON SURFACE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN/GLUCAN/HA BIOMATERIAL

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[ENGINEERING OF BIOMATERIALS 148 (2018) 9]

Introduction

Atmospheric pressure plasmas with their afterglow effect are often used for environmental and biological applications. Electromagnetic field, UV radiation, and share stress induced by the substrate gas flow can act on the sample in parallel to the chemical impact caused by reactive oxygen and nitrogen species generated in plasma. Thus, the synergetic effects of treatment can be expected. In surface processing, also in the case of biomaterials, which are not high temperature resistant, plasma is primarily used for surface cleaning, deposition, ablation or etching, surface activation and wettability control, crosslinking, and the formation of new functional groups [1-2]. The aim of this study was to evaluate the effect of plasma treatment on wettability and surface chemical composition of chitosan/β-1,3-glucan/HA biomaterial.

Materials and Methods

The chitosan/β-1,3-glucan/HA biomaterial was produced by mixing the liquid phase (the blend of 2.0 wt.% chitosan and 8.0 wt.% β-1,3-glucan) with 80 wt.% HA granules followed by thermal gelation for 20 min. at 90ºC and neutralization in NaOH solution [3]. Plasma was generated in a compact, AC powered glidearc reactor (GAD), which was operating at the atmospheric pressure and consisted of 2 diverging profiled cupper rod electrodes of 10 cm. The initial, smallest inter-electrode was settled as 3 mm [4]. Nitrogen was used as a substrate gas. Wettability as one of the important features describing the physicochemical surface properties of biomaterials was measured using Kruss DSA25E goniometer. Static and dynamic water contact angles (CA) were observed using static and dynamic sessile drop methods, respectively. ATR-FTIR analysis was performed to determine changes in surface chemical composition of the biomaterial.

Results and Discussion

After 16 s of GAD treatment, slight increase of hydrophobic properties was observed due to the initial behavior of polar groups present in the highly porous sample material. The observation of advancing water CA revealed slightly different surface behavior of plasma treated sample in comparison to the control: decrease to 20º after time and then its further penetration to the pores (FIG. 1 and FIG. 2). No significant changes in functional groups composition on the surface of the biomaterial was observed after 16 s of GAD treatment in nitrogen.

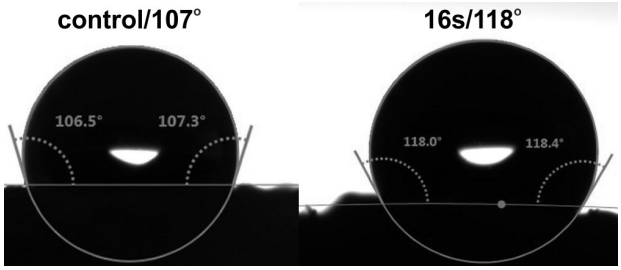

FIG. 1. Static sessile drop measurement of contact angle for GAD treatment.

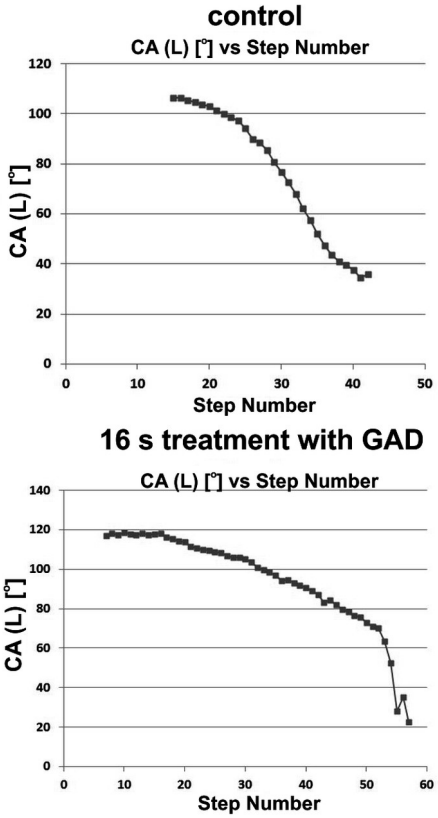

FIG. 2. Dynamic measurement of water contact angle for GAD treatment, substrate gas: nitrogen.

Conclusions

Obtained data demonstrated that plasma treatment of chitosan/glucan/HA biomaterial with the use of nitrogen as a substrate gas only slightly affects wettability and does not change surface chemical composition of the tested composite material.

Acknowledgments

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IMPACT OF HUMAN SERUM ON HAP-GLUCAN COMPOSITE

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[Engineering of Biomaterials 148 (2018) 10]

Introduction

Biomaterials for bone tissue regeneration, including polymer-based composites, are typically evaluated *in vitro* prior to the clinical trials. However, such composites tested *in vivo* may behave different due to the specific body conditions – for example, they may swell in acidified tissue liquids (due to the appearance of inflammation). This is likely for composite based on water-absorbing polymers, such as chitosan, starch or hyaluronic acid [1,2]. Such swelling of implanted biomaterials is likely to evoke side effects, for example to expand within bone defect. Massive swelling was observed for elastic HAp-glucan biocomposite for bone defects regeneration in inflamed (acidified) tissue [3]. To verify whether the composite swelling appears also in standard body environment, the composite was soaked in human serum of neutral pH. After the incubation, the changes in crucial physicochemical parameters of composite were evaluated.

Materials and Methods

Composite samples were synthesized as previously described [4] with a permission of Medical Inventi Ltd (owner of intellectual property for HAp/glucan composite). Composite samples (ø 5 mm and ø 13 mm) were sterilized in plastic/paper peel pouch (ethylene oxide method) and soaked in human serum of neutral pH (7.4) collected from healthy persons for 5 days at 37°C. Part of the samples was pre-soaked in Ringer solution for 30 min. Changes in weight and volume of samples in specified time points were measured. Then samples were subjected to evaluation of physicochemical parameters using different techniques (microCT, XRD, FTIR, mercury porosimetry, mechanical testing).

Results and Discussion

Relative weight and volume of composites presoaked in Ringer solution did not change significantly between the beginning and the end of soaking process. The changes varied maximum by 6% (relative weight) and 5% (relative volume). In contrast, more distinct changes in relative weight and volume of samples were observed for samples soaked exclusively in serum: maximum by 52% (relative weight) and by 16% (relative volume) (FIG. 1, 2). Microstructure, porosity, chemical composition and mechanical properties of the composite were not altered by neutral human serum after the incubation. The results suggest that pre-treatment in Ringer solutions prevents any undesired changes of biomaterial volume within the first period after the implantation.

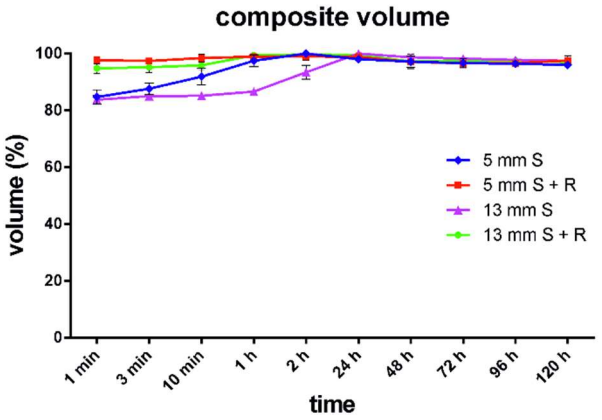

FIG. 1. Changes in composite volume (*source: L. Borkowski et al., J. Biomed. Mater. Res. Part B 2018, doi: 10.1002/jbm.b.34082*).

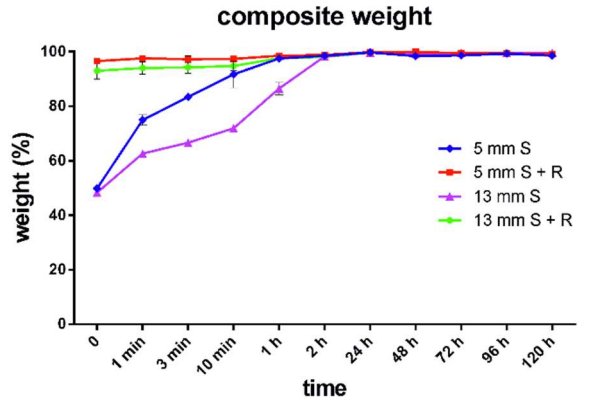

FIG. 2. Changes in composite weight (*source: L. Borkowski et al., J. Biomed. Mater. Res. Part B 2018, doi: 10.1002/jbm.b.34082*).

Conclusions

The results of HAp/glucan composite behaviour in media of neutral pH suggest that the composite swelling is size-dependent, time-limited (appear during up to 24 h of incubation) and that human serum penetrates the composite structure relatively slowly, in comparison with low-viscous media. Neutral pH of incubation medium allows to prevent the excessive increase of composite volume. Pre-incubation in Ringer solution (protein-free medium) protects the composite against undesirable swelling. Therefore, presoaking of the composite prior to the implantation (for example in saline or drug solution) is highly recommended to reduce the risk of post-operative side effects.

Acknowledgments

This study was supported by a DS2 grant (Medical University in Lublin, Poland). Authors want to express their gratitude to Medical Inventi Ltd (owner of intellectual property for HAp/glucan composite) for its permission to study the composite behaviour.

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DIALYSIS METHOD FOR β-1,3-GLUCAN GELATION PROVIDES HIGH BIOCOMPATIBILITY OF β-1,3-GLUCAN/HA BONE SCAFFOLD

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[ENGINEERING OF BIOMATERIALS 148 (2018) 11]

Introduction

Beta-1,3-glucan (curdlan) is a non-toxic, bacterial, and linear polymer which possesses ability to form firm and flexible gel [1]. It was shown that thermally obtained curdlan gel is a suitable component of bioactive bone substitute [2] as well as biocompatible bone scaffold [3]. The aim of this study was to evaluate whether dialysis method for curdlan gelation is suitable for fabrication of biocompatible and bioactive bone scaffold.

Materials and Methods

The β-1,3-glucan/HA scaffold (glu/HA D) composed of 8 wt.% curdlan and 80 wt.% HA granules was made via dialysis method against $CaCl_2$ as described in details in Patent No. P.415936 [4]. Microstructure of glu/HA D scaffold was visualized by SEM. The cell-biomaterial interactions were assessed by evaluation of osteoblast (hFOB 1.19 and MC3T3-E1 cells) viability, adhesion, and proliferation in direct contact with glu/HA D scaffold. In turn, bioactivity of glu/HA D scaffold was estimated by measurement of changes in ionic composition of culture medium as well as by evaluation of *in vitro* apatite-forming ability after soaking in SBF.

Results and Discussion

The cytotoxicity assay demonstrated that the viability of both hFOB 1.19 and MC3T3-E1 cells seeded on glu/HA D scaffold was high and exceeded 70% in comparison with control cells (TABLE 1). Moreover, it was proved that number of cells grown on the scaffold increased with time indicating that glu/HA D material promoted osteoblast survival and proliferation (FIG. 1). As revealed by ion reactivity test (FIG. 2), glu/HA D scaffold released huge amount of Ca^{2+} ions to the culture medium, what positively affected cell-scaffold interaction and also apatite-forming ability *in vitro*. SEM analysis (FIG. 3) demonstrated the occurrence of characteristic crystals on the glu/HA D scaffold already on the 14th day of experiment. EDS analysis (FIG. 3) confirmed results obtained with SEM and showed that observed layer was composed of calcium phosphate with Ca/P ratio ranging from 1.7-1.72, which is similar to Ca/P ratio in hydroxyapatite (1.67).

TABLE 1. Viability of osteoblast cells seeded on glu/HA D scaffold (assessed after 24-h culture).

	Viability [% of control ± SD]
hFOB 1.19 cells	71.30 ±1.82
MC3T3-E1 cells	87.72 ± 6.31

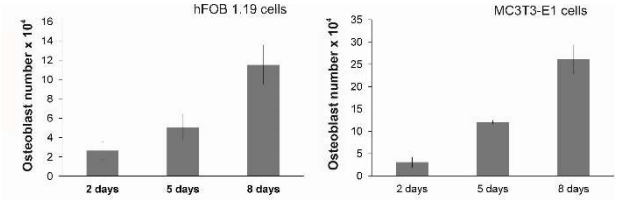

FIG. 1. Evaluation of osteoblast cells growth on glu/HA D scaffold.

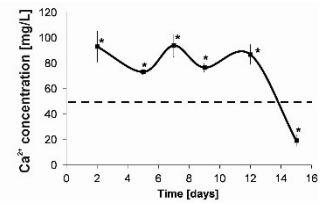

FIG. 2. Changes in Ca^{2+} concentration during 15-day of glu/HA D soaking in culture medium. Dotted line – ion concentration in fresh culture medium. *significantly different result compared to fresh medium (unpaired *t*-test, Graph Pad Prism 5).

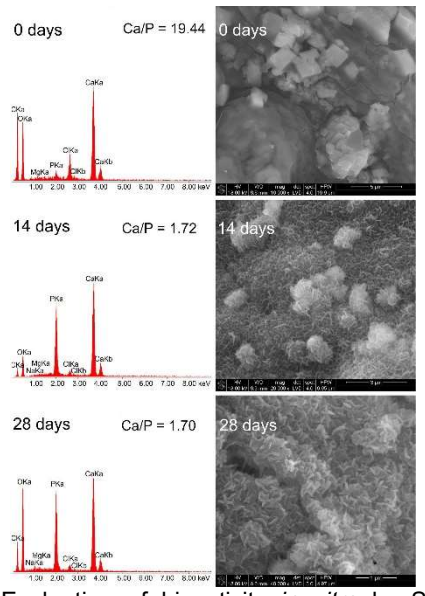

FIG. 3. Evaluation of bioactivity *in vitro* by SEM/EDS analysis during 28-day of glu/HA D soaking in SBF.

Conclusions

Within this study it was demonstrated that dialysis method for curdlan gelation may be successfully used for HA-based biomaterial fabrication. Produced glu/HA D scaffold releases huge amount of Ca^{2+} ions to the surrounding environment, what positively affects osteoblast viability, adhesion, and growth. Moreover, glu/HA D scaffold possesses ability to form apatite layer on its surface. Thus, considering biocompatible and bioactive properties of glu/HA D scaffold, it may be concluded that it is a promising biomaterial for bone tissue engineering.

Acknowledgments

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INFLUENCE OF INCUBATION TIME ON THE SURFACE OF NASAL SPLINT USED IN OTORHINOLARYNGOLOGY

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[ENGINEERING OF BIOMATERIALS 148 (2018) 12]

Introduction

The time of contact of biomaterials with patient environment has important influence on development of biofilm (bacterial adhesion) on an implant surface. The tests of biofilm formation on nasal splints used at the conclusion of nasal septal surgery to determine the optimal time frame for the removal of nasal packing material are important for safety of patients [1,2,4]. An analysis of levels of wettability and surface free energy become significant aspects in testing of biomaterials, from the point of view of bioactivity [6].

Materials and Methods

The object of the study was a nasal splint of Atos Medical implant used in procedure of stabilized the nasal septum. The changes of surface free energy (γ_S SFE), its components (dispersive γ_s^d, polar γ_s^p, γ_s^+ basic, γ_s^- acidic) by using analytical Owens-Wendt (OW) and van Oss-Chauhury-Good (vOCG) models were estimated [3]. Three measurements liquids: (W) distilled water (Poch S.A.), (D) diiodomethane (Merck sp. z o.o.) and (G) glycerin (Chempur) were used. The contact angle values were measured with the use of sessile drop method by the See System computer-based instrument produced with Advex Instruments. The volume of liquids drops was 0.5 µl, each test was repeated ten times in room temperature of 22±1°C. The samples of nasal splint were storage in closed containers by 30, 60 and 420 days in 0.9% NaCl solution (Polpharma) in 40°C temperature (in heat chamber Advantage-Lab E2 of Advantage-Lab), as simulated body conditions. The results were compared with reference sample (0 days incubation). The DLVO theory (Derjaguin-Landau-Verwey-Overbeek) was used to predict the interaction between implant (nasal splint) and patient [5].

Results and Discussion

The average values of contact angles (FIG. 1 with ±SD) were used to determine the SFE and its components by OW and vOCG models (TABLE 1).

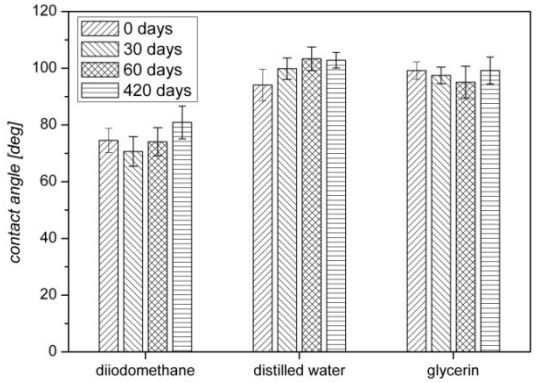

FIG. 1. The influence of incubation time on values of contact angle of silicone nasal splint.

The results showed an influence of incubation times on the values of contact angles. After 30 days of incubation the value of SFE was quasi-stabile, but dispersive component increased a little. It decreased over time, until exceeding the value of the left limit of nonadhesive (FIG. 2 – 420 days, blue dot in area medium of interaction). According to [6] the optimum surface free energy for which bacterial adhesion force is minimum can be derived by using the DLVO and for PTFE is 25-30 mN/m. Our values of SFE were quite similar to these given in [5].

TABLE 1. The influence of incubation time on values of SFE and its components mJ/m^2 of nasal splint.

days	M/(L)*	γ_S	γ_s^d	γ_s^p	γ_s^+	γ_s^-
0	OW/(W-D)	23,5	20,4	3,2	-	-
30		23,8	22,5	1,3	-	-
60		21,5	20,6	0,9	-	-
420		18,6	17,0	1,6	-	-
0	vOCG/ (W-D-G)	25,6	20,4	5,2	0,6	10,9
30		24,7	22,5	2,2	0,3	4,5
60		20,6	20,6	0,0	0,0	1,8
420		17,5	17,0	0,5	0,0	3,7

*M – method, L- liquids

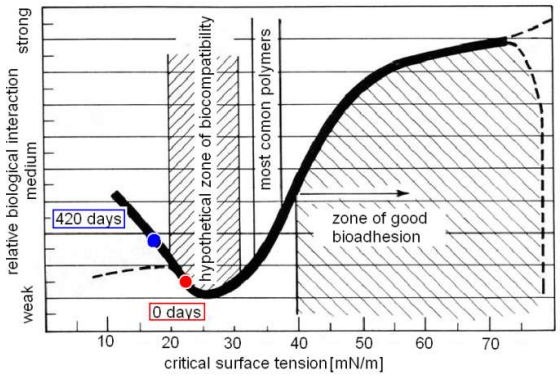

FIG. 2. Theoretical relative biological interaction.

Conclusions

The mechanisms adhesion of biofilm to laryngological material depends on many factors associated with a substrate, including SFE. This method allows to estimate a potential for reducing both bio- and mineral fouling [5]. The values of SFE and its components obtained by OW and vOCG methods were similar, but the vOCG method can be used for evaluating bacterial adhesion forces by DLVO method. Time of contact splint material with biological environment is very important issue. It could be concluded that extremal long-term simulation of contact nasal splint with body started the bioactivity process.

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INNOVATIVE MACROPOROUS CHITOSAN/AGAROSE MATRIX-BASED BIOMATERIAL FOR BONE TISSUE ENGINEERING APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 13]

Introduction

Scaffolds for bone tissue engineering applications should possess good biological and mechanical properties as well as high porosity, especially open and interconnected one. The porosity enhances adhesion, proliferation, and differentiation of osteoprogenitor cells/osteoblasts promoting faster rate of bone ingrowth [1]. The aim of this study was to develop novel macroporous chitosan/agarose matrix-based biomaterial for bone tissue engineering applications and to evaluate its basic structural, mechanical, and biological properties.

Materials and Methods

Porous biomaterial made of chitosan/agarose matrix and hydroxyapatite nanopowder (nanoHA) was fabricated using sodium bicarbonate as a gas-foaming agent and freeze-drying method. The mix of chitosan solution, agarose suspension, gas foaming agent and nanoHA was subjected to a high temperature and subsequently to freezing. Then, the sample was lyophilized to obtain highly porous structure of the biomaterial. The resultant scaffold, marked as chit/aga/nanoHA, was composed of 2% chitosan, 5% agarose, and 40% nanoHA.

Microstructure visualization. The porosity and surface of the biomaterial were determined by microcomputed tomography (μCT) and scanning electron microscopy (SEM), respectively.

Compression test. The Young's modulus value and compressive strength of cylinder-shaped samples were evaluated by Zwick Roell Z2.5 testing machine.

Cell culture experiments. The study was conducted using mouse calvarial preosteoblasts (MC3T3-E1 Subclone 4) obtained from ATCC. The cytotoxicity of the biomaterial was assessed according to ISO 10993-5:2009 by indirect test using fluid extract of the chit/aga/nanoHA scaffold. Cell viability upon exposure to the extract was determined by MTT assay. Cell spreading and morphology on the biomaterial was evaluated by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin. Stained cells were analysed under confocal microscope.

Statistical analysis. The unpaired t-test was carried out to assess statistical differences between control cells and the cells cultured in the presence of the extract. Statistical significance was considered at a probability with $p < 0.05$ (GraphPad Prism 5, Version 5.03 Software).

Results and Discussion

Microstructure analysis showed that developed biomaterial is characterized by rough surface, highly macroporous structure (total porosity approx. 50%, pore diameter > 100 μm), and relatively high interconnected and open porosity (FIG. 1). According to available literature, interconnected and macroporous structure of biomaterial provides good osseointegration, vascularization, and oxygenation of the implant *in vivo* [2]. However, the chit/aga/nanoHA material revealed low compressive strength and Young's modulus values, which were equal to 1.4 MPa and 13.98 MPa, respectively (TABLE 1). It is worth to emphasize that it is common phenomenon that introduction of high porosity into the biomaterials inferiors their mechanical parameters [3]. MTT assay revealed that chit/aga/nanoHA biomaterial is non-toxic to the cells because osteoblast viability was near 100% compared to the control cells. Moreover, confocal microscope observation confirmed non-toxic character of chit/aga/nanoHA since cells cultured on the biomaterial were well attached and spread (FIG. 2).

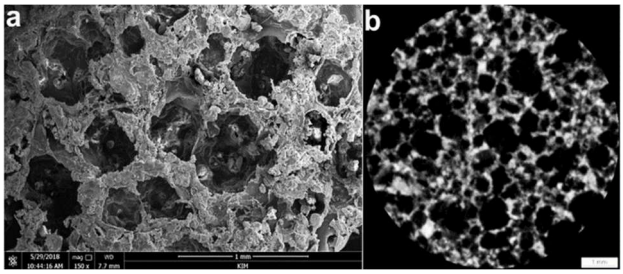

FIG. 1. Visualization of chit/aga/nanoHA microstructure: (a) SEM image of the surface, magn. 150x; (b) μCT cross-section presenting porosity of chit/aga/nanoHA.

TABLE 1. Mechanical properties of chit/aga/nanoHA.

chit/aga/nanoHA scaffold	
Compressive strength [MPa] ± SD	1.4 ± 0.18
Young's modulus [MPa] ± SD	13.98 ± 1.8

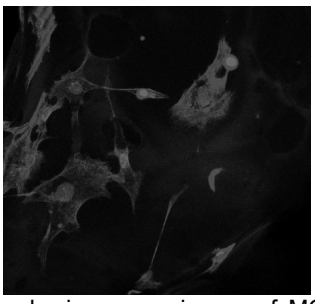

FIG. 2. Confocal microscope image of MC3T3-E1 cells cultured on the biomaterial, magn. 200x.

Conclusions

Obtained results demonstrated that simultaneous application of foaming agent and freeze-drying technique for biomaterial fabrication allows to gain highly macroporous, non-toxic, and supportive to osteoblasts growth biomaterial. Despite relatively poor mechanical properties of fabricated biomaterial limiting its application to non-load bearing implantation areas, the scaffold may be potentially used to generate living grafts under *in vitro* conditions.

Acknowledgments

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EVALUATION OF THE DIFFERENTIATION OF STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES OF ORTHODONTIC WIRES OF AISI 304 STAINLESS STEEL

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[ENGINEERING OF BIOMATERIALS 148 (2018) 14]

Introduction

Wires used for orthodontic arches play a very important role in the process of orthodontic treatment [1,2]. In combination with the lock attached to the tooth, they are to move and align the teeth along the set trajectories [3]. Wires of stainless steel, nitinol, beta-titanium and cobalt-chromium alloy are commonly used [4,5]. Each of the specified orthodontic wire types, due to its specific, desirable properties is used at different stages of orthodontic treatment. Wires of stainless steel are commonly used in orthodontics for several reasons: they are characterised with high resistance to corrosion, high strength and elasticity, formability and a possibility of obtaining defined properties through cold working and annealing during production process, as well as low cost of manufacturing [4,5].

Materials and Methods

Purpose of the research presented in the work is analysis of differentiation of the selected structural properties in the context of corrosion resistance of the orthodontic wires material. The object of the research were edge arches of the 0,016"x0,022" size made of the stainless steel type AISI 304, provided by two different producers. The research methodology involved analysis of chemical and phase composition of the tested alloy, microscopic tests with application of the light and electron microscopy methods, as well as electrochemical direct current measurements.

Results and Discussion

The chemical composition tests have shown that the orthodontic wires from various producers differ in contents of individual alloy elements, as well as they exceed the contents of permissible manganese and sulphur values allowed by the standard. However, it could be stated, that the information provided by the producers that the wires are made of stainless steel of the AISI 304 type are consistent with reality.

Evaluation of the metallurgical purity degree has shown considerable diversity in terms of deployment and appearance frequency of non-metallic inclusions in the orthodontic wires from different producers. The research has shown that presence of the non-metallic inclusions in the tested materials is equal to the standard No. 2, according to the ISO standard, which is unacceptable for materials applied in the living body.

Microstructure of the tested wires applied for orthodontic arches have shown appearance of strongly deformed structure of austenite (the fibrous texture along the drawing direction). Between strongly deformed grains of austenite the clear initial etchings around other microstructures have been observed. The XRD tests have shown occurrence of austenite in the microstructure of martensite induced by α' draft, as well as chromium carbide of the M23C6 type. The three-phase structure is particularly unfavourable due to the corrosion resistance, as presence of the M23C6 carbide in the tested material will favour the intercrystalline corrosion. Instead, presence of martensite, the ferromagnetic phase will adversely affect the body the orthodontic wires will be in, possibly causing magnetotropism of blood components and additionally be a source of corrosion.

The corrosion of orthodontic wires is closely related to the acidic environment of the mouth and the presence of fluoride ions, prophylactic agents and mouthwash solutions. Due to the thermic, microbiological and enzymatic properties aspects, the mouth environment is favourable in terms of biodegradation of metal and their alloys aspect, resulting in releasing metallic ions in the mouth. Along with the release of ions from metals or alloys the corrosion of orthodontic wires may lead to increase in surface roughness and their weakening, which can seriously impact the material strength, leading to mechanical damage or even fracture of the orthodontic materials. Based at the obtained test results of the orthodontic wires of the same geometry coming from two different producers it has been observed that in the environment of the Ringer solution they do not show the ability to passivate. At the polarization curves in the anodic area only the clear dissolution area is noticeable. At the surface of the orthodontic wires also the phenomena of pitting corrosion take place, which is confirmed by the course the anodic curves. Moreover, it has been found that content of non-metallic inclusions and carbides occurring in the material microstructure definitely lowers corrosion resistance of the material.

Conclusions

The research presented in the work have shown significant differentiation of structural and physical-chemical properties of the orthodontic wires of the AISI 304 type stainless steel. Despite the fact, that the tested arches were manufactured of the theoretically the same materials, but by different producers, they significantly differ with chemical composition, metallurgical purity, phase build and corrosion resistance. In addition, it is worth noticing that the tested materials, in terms of structure, do not meet the normative requirements obligatory for biomaterials.

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THE EFFECTS OF PRECONDITIONING ON TENSILE PROPERTIES OF PIG'S SKIN

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[ENGINEERING OF BIOMATERIALS 148 (2018) 15]

Introduction

Preconditioning is a procedure to load and unload the soft tissues several times before the data collection to achieve more repeatable testing results. Soft tissues e.g. tendons, skin or aorta are usually preconditioned before performing mechanical testing, such as tensile, creep, relaxation and hysteresis tests. This preconditioning significantly influences their mechanical properties [1-4]. This study investigates the influence of preconditioning on tensile properties of pig's skin tissue.

Materials and Methods

Skin tissue samples were taken parallel to the backbone from the back of 8-month old domestic pig, weighting about 100 kg. All samples had the same length of 100 mm and the width of 10 mm, however, these were of different thickness. The average thickness was 2.6 ± 0.2 mm. Samples were stored in the saline solution (0.9 %) at the temperature of 4°C no longer than 12 hours (fresh) before the test.

The uniaxial static tensile test was determined with the use of the MTS Insight 50 testing machine. The samples were mounted using scissor action grips with self-tightening and they were extended at a speed of 5 mm/minute at a room temperature of 22 ± 1°C. The initial gauge length was 50 mm. Registered force - elongation curves were recalculated into stress – strain curves. Before the tensile test, half of the samples were subjected to a preconditioning process. The preconditioning was performed in the load controlled experiment. The upper limit of load was taken to ensure that the strain remained within the linear region. The maximum load of each load-unload cycle was fixed at 5 N. Loading and unloading were repeated until the stress-strain loop of sample appeared to be periodic. It was after 3 to 5 cycles of loading-unloading for each sample. For each test at least 5 samples were taken for results analysis.

Results and Discussion

The exemplary stress-strain curves before preconditioning were shown in FIG. 1 and after preconditioning in FIG. 3.

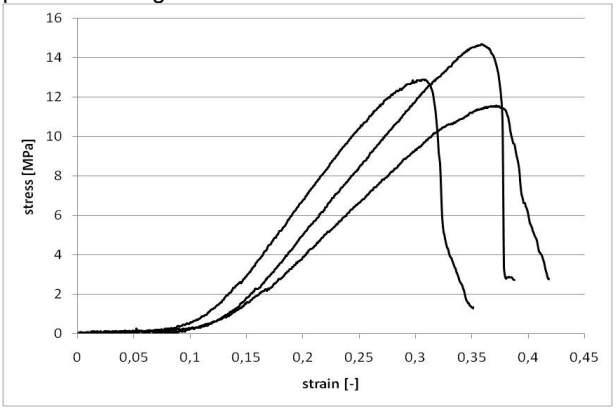

FIG. 1. The exemplary stress-strain curves before preconditioning.

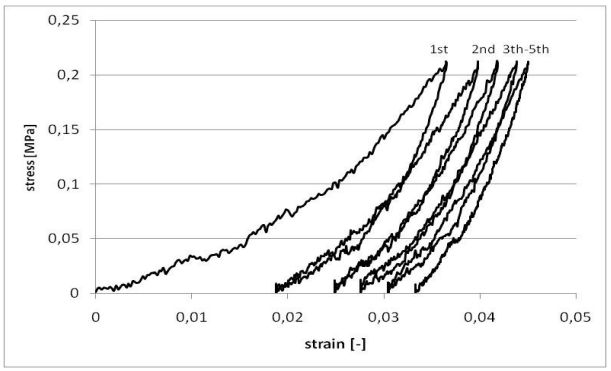

FIG. 2. The exepolary hysteresis loops registered during preconditioning.

Under the repeated cyclic loading stress-strain loops moved towards right and become repeatable, demonstrating preconditioning phenomenon of skin tissue (FIG. 2). However, preconditioning influenced on mechanical properties of skin (TABLE 1) and caused the increase of repeatability of results, it is difficult to clearly determine the impact of the preconditioning process.

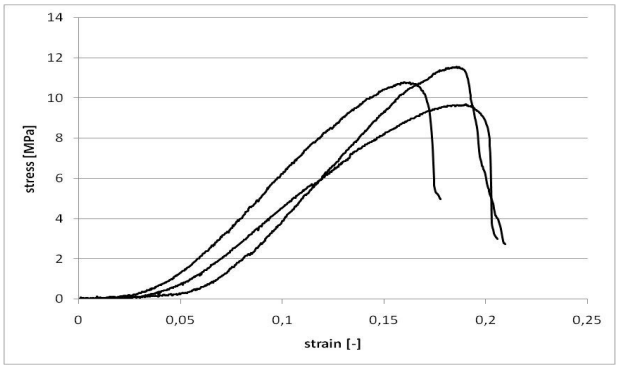

FIG. 3. The exemplary stress-strain curves after preconditioning.

TABLE 1. The average values of tensile strength (σ_{max}), strain at maximum force (ε_M) and Young's modulus (E)

	σ_{max} [MPa]	ε_M [-]	E [MPa]
before preconditioning	13.0±1.6	0.34±0.30	89.1±14.5
after preconditioning	10.1±0.6	0.18±0.02	122.5±18.5

Conclusions

The preconditioning in repeated cycles is common feature of skin tissue but still there are no exact procedure of it. According to Fung, the tissue should be preconditioned at the same stress levels as the subsequent testing. These obviously cannot be achieved in case of loading at large deformations so still further research is needed.

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SELENIUM – ENRICHED BRUSHITE: A PROMISING MATERIAL FOR POTENTIAL USE IN BONE IMPAIRMENTS TREATMENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 16]

Introduction

Bioactive and biocompatible, calcium phosphates (CaP) are widely applied biomaterials in bone tissue and dental surgery, serving as bone fillers, coating materials and drug delivery system matrices [1]. One of them, dicalcium phosphate dihydrate (DCPD), described with the formula $CaHPO_4 \cdot 2H_2O$ (known as a mineral brushite) exhibits relatively high solubility [2] and is considered to be an intermediate phase in the processes of bone mineralization and enamel dissolution.

The solubility of potential bone-substituting materials has an impact on the regeneration of mineralized tissue and the release rate of therapeutic agents, i.e., foreign ions or drugs possibly introduced into the crystal lattice of CaP. Introducing foreign ions is one of the potential ways to improve the biomaterial's properties. For instance, silicon may enhance its bioactivity, silver can provide it antibacterial activity, while selenium usually serves as internal anticancer agent [3-4].

Apart from taking part in oxidative stress protection, affecting positively the immune system and being essential for bone turnover, selenium, as it was mentioned above, exhibits an anticancer activity. Among different selenium species, selenites (SeO_3^{2-}) revealed the most significant effectiveness of this kind [5].

To the best of our knowledge, there have been no published studies on DCPD containing selenium. Due to this and the reasons mentioned above, in this work, Se-doped brushite was synthesized using a standard, wet method and then physicochemically examined.

Materials and Methods

Both samples of pure brushite (Bru) and selenium-doped brushite (Se-Bru) were prepared using a wet precipitation method. To obtain selenium-modified brushite (Se-Bru) the reagents: $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2HPO_4$ and Na_2SeO_3 were weighed out so that the molar ratio of Ca/P+Se was close to 1.0 and then dissolved in distilled water. The water solutions of phosphate and selenite were instilled into the calcium solution. The precipitation process was carried under permanent stirring. Once adjusting the pH to about 6, the stirring was continued for one hour. Afterwards, the precipitate was left for 24-hour aging and subsequently filtered and washed out with distilled water. Finally, the precipitate was dried at a temperature of 90ºC for 24 h. The route of the synthesis of pure brushite (Bru) was technically the same. The only difference was that there was no selenium source among the reagents, which were weighed out so that the Ca/P ratio was ca. 1.0.

The dried powders were homogenized in mortar and characterized using following methods: SEM microscopy, PXRD, FT-IR, ssNMR and ICP-MS.

Results and Discussion

The SEM microphotographs (FIGs. 1A and 1B) proved that the investigated samples differed significantly in morphology. The elongated and plate - like shaped, crystals of pure Bru (FIG. 1A) possessed a diameter and length of about 10 and 20-30 μm, respectively. In turn, Se-Bru sample exhibited a rod-like morphology (FIG. 1B) with the crystals of the diameter of 5-7 μm and length above 25 μm. Unlike Se-Bru crystals, Bru ones were strongly agglomerated.

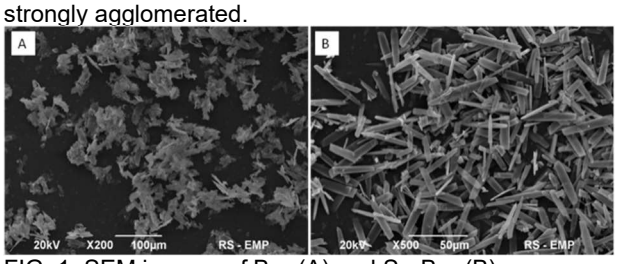

FIG. 1. SEM images of Bru (A) and Se-Bru (B).

Analysing the diffractograms of both samples, all of the reflections were assigned to the brushite monoclinic structure (JCPDS 09-0077). No impurities were detected. A visible reduction of the relative intensity of (020) and (040) reflections of the Se-Bru sample was observed. In the Se-Bru diffractograms, the reflections mentioned above exhibited a slight difference in position, which indicates that introducing selenite affected the crystallinity and crystal morphology of the sample [6]. Furthermore, the significant increase of the *a* parameter accompanied by the simultaneous decrease of the *c* parameter in the Se-Bru sample proves that selenite ions were incorporated into the crystal structure (TABLE 1) [7].

TABLE 1. Various samples' parameters determined from the PXRD diffractograms.

Parameters	Bru	Se-Bru
Phase composition	**100 % DCPD**	**100 % DCPD**
Unit cell parameters		
***a* (Å)**	**5.915**	**6.238**
***b* (Å)**	**15.12**	**15.16**
***c* (Å)**	**6.242**	**5.806**
***β* (°)**	**116.4**	**116.4**
Volume ($(Å)^3$)	**500.2**	**491.7**

The selenium content in the Se-Bru sample was measured using the ICP-MS method. The concentration of Se was equal to 0.67 ± 0.03 wt%.

Conclusions

In the present study, DCPD containing 0.67 wt% of selenium was synthesized. Future investigations concerning selenium release and *in vitro* studies on its biological properties are in progress and will contribute to the full evaluation of obtained material.

Acknowledgments

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ANTIMICROBIAL ACTIVITY OF MW PACVD +R MODIFIED DETONATION NANODIAMOND PARTICLES (DND)

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[ENGINEERING OF BIOMATERIALS 148 (2018) 17]

Introduction
Advances in nanotechnology have led to the development of many microbicidal nanoparticles with anti-biofilm effects. This is one of the applications of nanoparticles resulting from their properties. Nanoparticles can concentrate drugs on their surface, causing additional effects to improve their ability against bacteria. Nanodevices (NDs) are one of the most promising materials for biomedical applications. Our results can therefore be a step forward in the development of alternative antibiotic-based strategies aimed at bacterial infections.

Materials and Methods
In this article, we will explain the effect of modified detonation diamond powder (MOD-DND) in the MW-PACVD + R reactor on the viability of bacteria on Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria.

Results and Discussion
We show that while MOD-DND particles are nontoxic to both pathogens, they show significant antibiophilic activity. The presence of MOD-DND particles reduces the formation of biofilm more efficiently than free menthol, unmodified oxidized NDs and ampicillin, a commonly used antibiotic.

Conclusions
The Introduction should introduce the background to the work that has been carried out. It should contain citations to the key literature to support this rationale and should lead to a clearly stated hypothesis or set of objectives.

ELECTROSPINNING OF BIO-NANO-CELLULOSE (BNC) NANOFIBERS EQUPMENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 18]

Introduction
Electrospinning is a method of obtaining nanofibers from a liquid polymer or its solution, using high voltage (<10 kV). The polymer under the influence of electric current takes the shape of the so-called fiber and moves towards the electrode with the opposite charge. In our studies, we obtained homogeneous and simple fibers. The multitude of meters that we can control during the electrospinning process allows us to obtain from the same starting material nanofibres with different properties. Unusual possibilities arise through the use of additional surface treatment of nanofibres by ionic techniques.

Materials and Methods
Electro spinning is a method of obtaining polymeric threads, both natural and synthetic. Although this method has been known for over fifty years, only the last decade has brought significant progress in this field [1,2]. This technique allows you to control the process to achieve the best results (thread thickness, pore size). Polymer threads are formed by applying high voltage to the solution. The drop thanks to the surface tension forces stays on top of the feeder needle. High voltage causes the disappearance of these forces and gradual extension of the drop. The surface of the solution at the end of the drop takes the shape of a so-called Taylor's cone. Further increase in potential causes the critical value to be exceeded and the jetty to be launched. It gradually solidifies and settles in the form of threads on grounded ground.

Results and Discussion
We show that the specialized equipment for the electrospinning stand for nanonics is a device that gives new light in the field of industrial-scale production of modern, biodegradable materials based on BNC. The studies carried out show a varied SEM morphology and developed specific surface area. In addition, analyses were performed for antibacterial activity.

Conclusions
Electrospinning technology makes it possible to obtain thin natural polymer fibers (e.g. collagen, nanocellulose) as well as synthetic fibers that can find numerous applications in many areas of science.

MATERIAL ENGINEERING IN CARDIOVASCULAR RECONSTRUCTION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 19]

Abstract

Reconstruction of the cardiovascular system: The aim of the work is to minimize thrombus formation life threatening in the pulsatile heart assist chamber, by the use of a new biomimetic heart valve made on the basis metal-polymer composites. The heart prostheses are dedicated to support the heart of patients with late heart disease failure. They are dedicated to therapy related to self-regeneration or as a bridge for transplantation. In the future, they will be able to help treatment with gene therapy for myocardial infarction. Systems with valves mechanically generate plaque activation by shear stress. This is due to the narrow gap that is between the petal and the ring. Composite materials and a new valve design can minimize this problem. Injected polyurethane with a metallic, titanium bonded insert optimal micro-scale flexibility with macro-stiffness for ensuring appropriate mechanical functions of the valve.

Acknowledgments

The works were carried out as part of the projects: M-ERA.NET/2014/01/2016 (bioVALVE).

CYTOTOXICITY OF HYDROXYAPATITE DOPED NANOPARTICLES ON OSTEOSARCOMA

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[ENGINEERING OF BIOMATERIALS 148 (2018) 20]

Introduction
Hydroxyapatite nanoparticles have recently been proposed as anticancer drug. Their cytotoxicity is associated to the sudden increase in calcium and phosphate ions inside cancerous cells following nanoparticle degradation. However, before nanoparticles (NPs) can enter the cell they first interact with the cell culture medium and the cell membrane and this could already alter cell behaviour. The present work seeks to disclose the contribution of each effect on cell cytotoxicity to have a better understanding of the mechanisms behind the use of NPs on cancerous cells.

Experimental
Hydroxyapatite doped nanoparticles were prepared by neutralisation of calcium hydroxide with phosphoric acid. Magnesium chloride was added in the reaction vessel to obtain magnesium doped nanoparticles. Cytotoxicity studies were performed assessing cell morphology and measuring the lactate dehydrogenase activity of cells in various scenarios: a) upon supplementing NPs on cells (direct contact), b) upon supplementing the NPs in inserts to avoid direct contact with cells but allowing any exchange of ions and molecules across the insert membrane (indirect assay), and c) by culturing cells on disks made compacting NPs to favour cell membrane interaction but avoiding internalisation.

Results and Discussion
The study demonstrated that cell viability was not affected when NPs were placed inside inserts proving that any exchange of ions between NPs and the medium was not toxic to cells. In addition, direct seeding of cells on top of disks made of compacted nanoparticles showed excellent cell adherence and spreading proving that they were not cytotoxic. However, when NPs we added in the cell culture media in direct contact with the cells, these died in less than 3 h due to NPs internalisation.

Conclusions
The results obtained in this work highlight the importance of NPs internalisation above other potential mechanisms of interaction and reinforces the need of improving internalisation in views of using these NPs for cancer treatment.

Acknowledgements
European Union and Spanish Government are acknowledged for PCIN-2017-128 project. MAT2015-65601-R project funded by the Spanish Government and the European Regional Development Funds. Catalan Government for 2017SGR1165 funds. MPG acknowledges the ICREA Academia award by the Generalitat de Catalunya. ME is thankful for the Serra Hunter fellow position, and CC for the Ramon y Cajal Fellowship.

CATALYTIC PRODUCTION OF HYDROXYL RADICALS UNDER PHYSIOLOGICAL CONDITIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 21]

Introduction

Reactive oxygen species (ROS) including superoxide ($\cdot O_2^-$), ozone (O_3), atomic oxygen (O), hydrogen peroxide (H_2O_2), hydroxyl radical (HO·) are known to have significant influences in biological systems [1,2]. Indeed, as by-products of normal intracellular metabolism, they play key physiological roles in cells and tissues and their controlled production could favour cell proliferation or differentiation [1,2] and therefore tissue regeneration. A way to control their production and to favour their reaction with bone (stem) cells would be to embed HO· radical production catalysts inside smart scaffolds. However, it is difficult to control catalysts size and structure within it. Our approach is then to incorporate iron catalysts in mesoporous silica particles, which could be embedded in smart scaffolds for bone regeneration. Indeed, inorganic nanoparticles incorporated in polymeric scaffold lead to their improvement [3]. The silica particles studied here will have several other useful functionalities, such as contrast agents and stimuli-responsive drug delivery.

In this study, the preparation and characterization of iron oxide catalysts on mesoporous silica particles to produce HO· radicals from H_2O_2 under physiological conditions is presented. It is shown that H_2O_2 can be efficiently converted into HO· radicals with such nanoparticles and the quantity of HO· radicals generated has been quantified and correlated to the catalysts characteristics.

Materials and Methods

Spherical MCM-48 particles were synthesized according a known procedure [4]. Hydrophobic magnetite nanoparticles (MNPs) were synthesized by co-precipitation and then coated with oleic acid [5], or by thermal decomposition of iron-oleate complex [6]. Hydrophilic MNPs were synthesized by using a 'two-step method' [7], where the MNPs are first synthesized and washed before being mixed with citric acid. Citrate-stabilized hydrophilic MNPs were also obtained following the procedure reported by Yang *et al.* [8]. The MCM-48 decoration by the MNPs was performed by first modifying the MCM-48 surface as described by Stoeva *et al.* [9], and the MNPs surface as described by Kim *et al.* [10], before reacting them together. MCM-48 decoration was also realised by synthesizing the NPs in its presence: MCM-48 (0.27 g) was added to $FeCl_3 \cdot 6H_2O$ (70 mg) and $FeCl_2 \cdot 4H_2O$ (26 mg) in 3.5 mL of deionized H_2O and stirred in an Ar atmosphere for 2 h. The mixture was heated to 90°C followed by rapid addition of ammonia solution (1 mL, 14 wt.%), and heating for a further 90 min. The mixture was cooled to RT and the solid isolated by filtration, washed with deionized H_2O and acetone, and dried under vacuum. A third way was impregnating MCM-48 with an aqueous solution of $Fe(NO_3)_3 \cdot 9H_2O$, under incipient wetness conditions (IWI). The impregnated powder was dried at 100°C (12 h), calcined at 500°C (5 h) and then reduced under hydrogen at 400°C. The powder catalysts were analysed by TEM with a LEO922 Omega Energy Filter Microscope operating at 120 kV. Room-temperature fluorescence spectra were recorded on a Varian Cary Eclipse instrument (excitation wavelength 346 nm).

Results and Discussion

The MCM-48 synthesised displayed a ~1278 m²/g specific surface area and homogeneous spherical structure (particle diameter between 270 and 530 nm).

Different approaches were used to decorate MCM-48 with magnetite nanoparticles (MNPs). First, hydrophilic or hydrophobic MNPs (~10 nm) were synthesized according to various methods and anchored post-synthetically or prepared concomitantly to MCM-48 synthesis. Second, MCM-48 was also impregnated with a solution of $Fe(NO_3)_3 \cdot 9H_2O$ followed by calcination/reduction. The obtained Fe_xO_y/MCM-48 composite materials were characterized and then tested for the production of HO· radicals from H_2O_2. These tests were carried out in the presence of coumarin to follow the production of HO· radicals by fluorescence spectroscopy (FIG. 2).

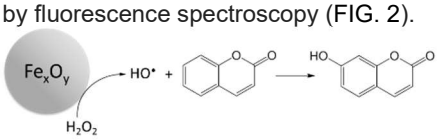

FIG. 2. Production of HO· from H_2O_2 on iron oxide catalyst and reaction between HO· and coumarin forming fluorescent 7-hydroxycoumarin.

This allowed us to discriminate our materials and select the most promising, which are those with smallest Fe_xO_y particles (< 2 nm). These small sizes are associated with amorphous particles. FIG. 2.a shows the results obtained with Fe_xO_y/MCM-48 presenting different iron loadings, which demonstrates the ability of our materials to produce HO· radicals. The TEM image visible in 2.b corresponds to the catalyst with the lowest iron loading.

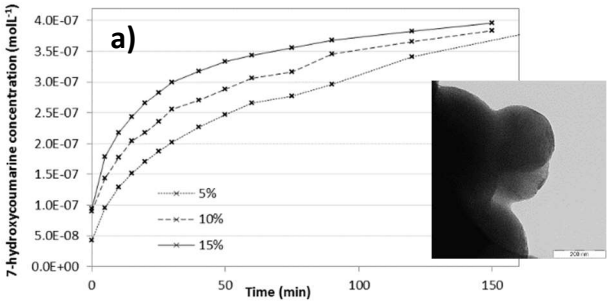

FIG. 2. (a) Kinetic and (b) TEM analysis of Fe_xO_y/MCM-48 catalyst with different wt.% iron loadings.

Conclusions

We have shown that iron-based catalysts are efficient for the production of HO· radicals from H_2O_2 in water at room temperature and we were able to follow this production over time by fluorescence spectroscopy.

Acknowledgments

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THE INFLUENCE OF THE SURFACE TOPOGRAPHY ON THE CELL-DIFFERENTIATION EFFECT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 22]

Introduction

The plasticity of non-embryonic stem cells and their potential to de-differentiate provides new therapeutic strategies in regenerative medicine. Derived from human endothelial cells, differentiated progenitor cells provide reliable, reproducible and physiologically appropriate source of cells for the treatment of vascular disease, atherosclerosis, coronary heart disease, hypertension and inflammatory diseases. The aim of the work was to explore and characterize the influence of defined surface topography of thin haemocompatible thin films (a-C:H) for effective capturing progenitor cells from whole human blood for differentiation into vascular endothelium.

Materials and Methods

200 nm thin a-C:H films (amorphous hydrogenated "diamond-like" carbon) were deposited by magnetron sputtering of a pyrolytic carbon target in ($Ar+C_2H_2$) atmosphere on soft, 1 mm thin thermoplastic polyurethane foil substrates. Homogenous surface topography of the films with uniaxial wave structure was achieved by pre-straining of the foils before deposition and release of the strain afterwards, which leads due to different elastic moduli (E) of substrate and film to the formation of "wrinkles" [1-4].
The interaction of endothelium progenitor cells (i-Cells) with the defined topography, mimicking tissue niches of the ECM was investigated by a VascuLife VEGF Medium Complete Kit (LifeLineCell Technologies, US).

Results and Discussion

The degree of differentiation was tested using anti-CD62E (E-selectin) and anti-CD31 (PECAM-1) antibodies. The results are presented in FIG. 2: PECAM-1 positive cells (yellow) indicate diversification into endothelial cells, E-Selectin (red) leukocyte-endothelial cell adhesion molecules, ZO-1 staining (green) tight intercellular junctions, and blue staining the cell nuclei (FIG. 1).

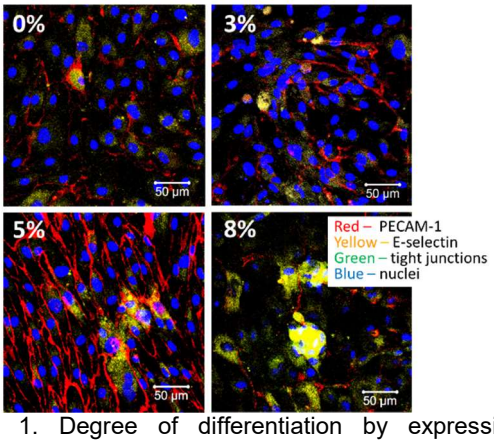

FIG. 1. Degree of differentiation by expression of PECAM1 (red), Selectin-E (yellow), ZO-1 (green) and DAPI (blue, indicating cell nuclei).

As visible, especially the 5% pre-straining result in a very regular network of PECAM-1 expression (leukocyte-endothelial cell adhesion molecules). There is a probability that deformation limit exists between 5% and 8% strain. In the case of the 8% deformation, the degree of cell differentiation and the likelihood of the appropriate cell-cell interactions formation that indicate the cell monolayer creation is weakened.

Conclusions

Summing up, the influence of the surface tomography on the cell-material interaction was observed. The surface tomography could influence on the cell differentiation effect as well as the monolayer formation. Both phenomena could have the significant meaning on potential use of biomaterial in cardiovascular regeneration. At the work, the biomaterial should not come into direct contact with blood. Biomaterial should create an appropriate environment for the self-formation of the endothelium. Endothelium is the natural layer that is able to self-regulate the clotting process. Therefore, the appropriate differentiation of progenitor cells into endothelium and the generation of appropriate cell-cell interactions is crucial. The paper shows the influence of surface topography, adequately formed on the degree of differentiation.

Acknowledgments

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COLLAGEN, SILK FIBROIN AND CHITOSAN BASED MATERIALS FOR BIOMEDICAL AND COSMETIC APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 23]

Introduction

Collagen is widely used for production of 3D sponges, wound dressings and scaffolds for biomedical applications [1]. It is also widely used in cosmetic preparations [2]. Due to high price of selected types of natural collagens there is a need to modify collagen to decrease the price, however, in the same time, to maintain the properties of this biopolymer. The blending of collagen with other water soluble polymers can lead to preparation of new materials suitable for biomedical applications [1,3]. In this work ternary blends based on collagen, chitosan and silk fibroin were prepared. The miscibility study was performed by viscometric measurements and calculation of parameters of interaction between polymers [4]. The miscibility can be estimated by determining the experimental parameters of the mixture and comparing them with the ideal parameters (calculated ones) [1,4]. The miscibility is the key for creating an ideal, homogeneous mixture. It gives the possibility to obtain a homogeneous material characterized by good mechanical properties. New materials based on the blends were turned into thin films and 3D sponges. The properties of the materials were studied.

Materials and Methods

Collagen (Coll) was obtained in our laboratory from tail tendons of young rats. Chitosan (CTS) was supplied by the company Sigma-Aldrich (Poznan, Poland). The deacetylation degree (DD, %) of CTS was 78%, and the viscosity average molecular weight was 0.59×10^6.
Silk fibroin (SF) was obtained from *Bombyxmori* cocoons in our laboratory following the method described by Kim et al. with slight modifications [5].
Collagen, silk fibroin and collagen were mixed together in appropriate weight ratio. All mixtures of polymers were placed in polystyrene container and frozen. 3D scaffolds were obtained during the lyophilisation process for 2 days. The structure of the blends was evaluated by attenuated total reflection infrared spectroscopy. The size of pores and their distribution were analyzed based on Scanning Electron Microscope (SEM) pictures. Surface properties of thin films were analyzed by AFM and contact angle measurements.

Results and Discussion

Viscosity measurements and IR spectroscopy showed that between components of the blend there are interactions. Strong interactions between the polymer blend components and the solvent were found by viscometric method. Depending on the weight ratio the ternary mixtures were classified as miscible and immiscible systems. According the structure of single biopolymers the interactions are due to hydrogen bonds formed between chemical moieties of polymers. After solvent evaporation from the polymer mixture thin films were obtained. The films show hydrophilic character. After lyophilisation process the 3D porous sponges can be obtained (FIG. 1).

FIG. 1. Scaffolds made from the blend of Coll and SF.

The cross-section of example of 3D sponge is shown in FIG. 2. As one can see the size of pores is irregular.

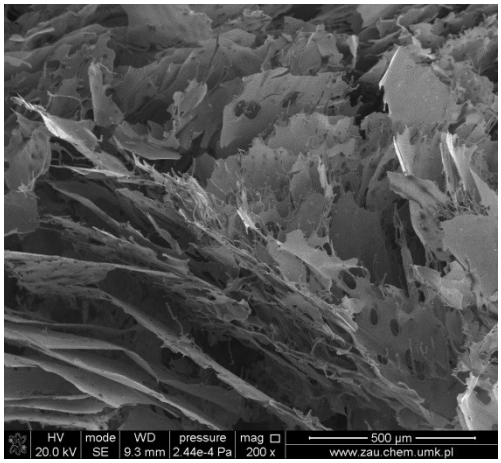

FIG. 2. The cross-section of collagen/silk fibroin blend liopilized into 3D sponge.

Film-forming properties of the blend are suitable for cosmetic applications. Mechanical properties and surface properties of new materials after covering the hair were studied. The adhesion of polymeric blend to the hair was very good.

Conclusions

Strong interactions between three components in polymer blend can lead to new material. The modification of biopolymer properties is a consequence of the strong interaction between the polymeric components. Biological properties of new materials should be studied

Acknowledgments

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STRUCTURAL VARIABILITY OF LYOPHILIZED COLLAGEN-BASED SCAFFOLDS: MICRO-CT 3D ANALYSIS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 24]

Introduction

Bone tissue engineering aims at regeneration of bone defects by application of biomaterials [1]. Structural parameters (e.g. porosity) are expected to have significant influence on scaffold-tissue interaction [2]. Lyophilisation is frequent fabrication method of tissue engineering scaffolds [3,4]. Micro-CT is an emerging non-destructive imaging method applicable for scaffolds structure evaluation [5]. The resulting 3D structure depends on lyophilisation parameters (e.g. temperature). Variability of structure in scaffolds prepared via the same procedure is not well described to date. Aim of this project is to assess the most convenient fabrication method for preparation of homogeneous 3D structure and evaluate its variability, which may limit its application in bone surgery.

Materials and Methods

Collagen-based scaffolds were prepared by means of lyophilisation using 3 different temperatures (-30°C, -80°C, -190°C; in each group were 20 specimens of cylindrical shape, h=7mm, d=6mm). Each specimen was micro-CT scanned (Bruker micro-CT SkyScan 1272, Belgium, Kontich) in air mounted on specimen holder-with following parameters: 4 um pixel size, frame averaging 5, no filter, scanning time approx. 1 hour. Image data were reconstructed and structural parameters (Structure separation, Structure thickness, Porosity, Object surface density) were evaluated by means of 3D analysis using Bruker software. Pore size was analysed based on sphere-fitting algorithm. Results were statistically evaluated (Kruskal-Wallis test, Bonferroni procedure, statistical significance was accepted at p≤ 0.05).

Results and Discussion

Specimens were visualized by virtual sections (FIG. 1) and 3D images.

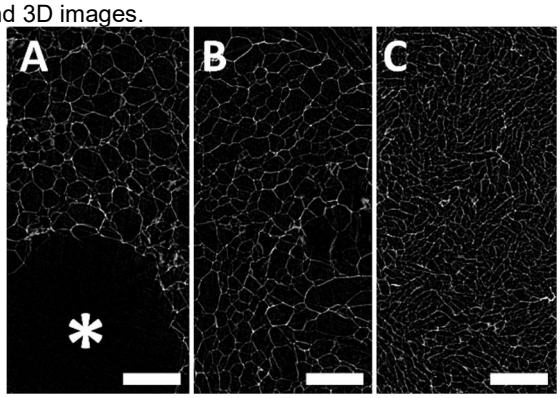

FIG. 1. Micro-CT 2D visualizations: A) -30°C; B) -80°C; C) -190°C. Differences in structure are clearly visible; large pore is shown in A(*). Scale-bars = 500 μm.

Pore size (Structure separation) was significantly reduced with lower temperature as was expected (FIG. 2-left). Thickness of pore walls (Structure thickness) was highest in -30°C (16.6 μm), both groups -80°C and -190°C presented lower values (approx. 16.1 μm). Porosity presented the highest values in -80°C (FIG. 2-right). Since closed porosity was below 0.01% in all specimens, open porosity was considered as total porosity. Object surface density was highest in -190°C (39 mm^{-1}), both groups (-30°C, -80°C) presented lower values (approx. 22 mm^{-1}).

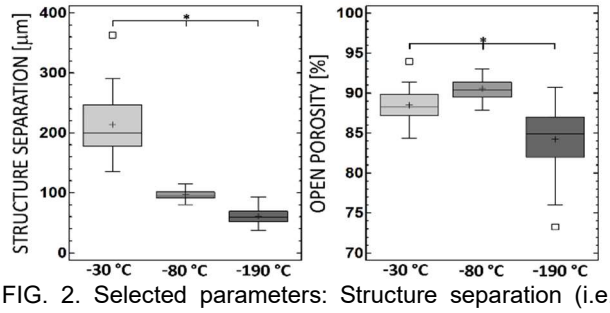

FIG. 2. Selected parameters: Structure separation (i.e. pore size) and Open porosity value in all groups. * denotes statistical significant differences, p=0.05.

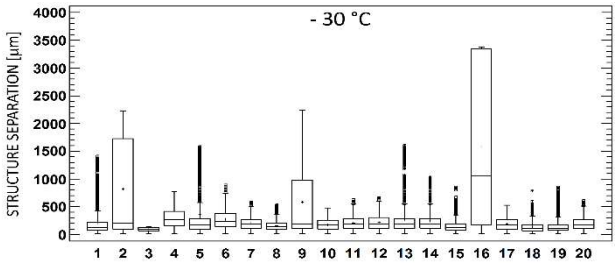

FIG. 3. Structure separation (Pore size) distribution in each specimen of -30°C group. 7 specimens present pores with diameter > 1 000 μm.

Variability of pore sizes among all specimens in each group was evaluated and significant differences were found. Variation coefficients (the ratio of the interquartile range to the median) were: -30°C=92.9%, -80°C=33.3%, -190°C=50%. Some specimens in -30°C group (FIG. 4) contained extensively large pores (see FIG. 1A*), which may limit application of these biomaterials.

Conclusions

Temperature of lyophilisation influences tissue engineering scaffolds structure. The most convenient mean pore size regarding recommended values presented -30°C group. However, due to its high variability, its application may be limited. Open porosity, important parameter, was highest in -80°C specimens. In general, the most predictable fabrication outcomes present lyophilisation at -80°C. In general, lyophilisation in -80°C was the most convenient fabrication method.

Acknowledgments

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OPTIMIZATION OF IN VITRO CELL CULTURE CONDITIONS FOR HUMAN MESENCHYMAL STEM CELLS OF DIFFERENT ORIGIN FOR APPLICATIONS IN TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 25]

Introduction

Mesenchymal stem cells (MSCs) are multipotent cells derived from different body tissues. They possess several features, such as robust differentiation capacity and immunosuppressive properties, which make them one of the most promising cell types for potential applicability in tissue regeneration. Their derivatives such as extracellular vesicles (EVs) also very promising due to the fact that they carry bioactive proteins and small nucleic acids such as mRNA and miRNA, which may have positive impact on different functions of target cells. Unfortunately, MSCs are routinely cultured in conditions containing fetal bovine serum that is a rich source of proteins and EVs of animal origin, which may be harmful for patients. If we want to transfer MSCs and MSCs EVs technology to the clinic we need to look for better culture conditions that support robust cell growth but are safe for humans.

Thus, the aim of this study was to select the most optimal serum-free, xeno-free culture medium for harvesting safe MSCs and MSC-EVs for the purpose of regeneration.

Materials and Methods

Human umbilical cord-derived MSCs (UC-MSCs) and adipose tissue-derived MSCs (AT-MSCs) were cultured in several different media. Cell proliferation, viability, metabolic activity (ATP concentration), multiantigenic phenotype (flow cytometry), and differentiation potential as well as senescence rate were measured after specified number of passages. mRNA levels for genes connected with differentiation, and cells secretome were analysed. MSC-EVs were isolated from conditioned media by ultracentrifugation at 100000g and characterised according to International Society for Extracellular Vesicles guidelines. Phenotype and transcriptome analysis of EVs, was performed as well as functional studies.

Results and Discussion

Our results indicated differences between MSCs cultured in the tested media including differences in proliferation rate, metabolic activity, longevity and gene expression levels that allowed for selection of most optimal one. We also observed that MSC-EVs were enriched in transcripts crucial for stimulation of cell differentiation.

Conclusions

Our data allowed us to choose most optimal media for MSCs culture and MSC-EVs harvest for purposes of tissue regeneration. However, further experiments are required for transfer of these results into clinic.

Acknowledgments

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BIOMECHANICAL ANALYSIS OF PLATES USED FOR CHEST TREATMENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 26]

Introduction

Deformation of the front chest wall has been an important therapeutic problem for years. In 1997, Donald Nuss presented a new surgical method, which consisted in introducing into a previously created tunnel between the pericardium and the anterior surface of sternum a properly shaped plate, and rotating it in order to elevate the sternum. The method does not require cutting or resection of osteocartilaginous parts [1]. This technique proves that it is possible to perform a surgical procedure correcting this defect in a way less traumatic for patients, and to accelerate the healing and rehabilitation processes [2], [3]. The results of clinical trials have been very positive so far. Single cases of plate rotation, leading to destabilization and pains, have been observed during loading of the sternum. The results of previous experimental research by the authors [4], concerning analysis of the bone tissue – implant system introducing changes in the plate structure and using additional bars preventing the plate rotation. To facilitate the assembly of the entire system in the plate, additional holes were made to allow the introduction of surgical instruments. For such a prepared model, numerical analysis was performed focusing on the values of reduced stresses occurring in system elements.

Materials and Methods

Geometrical models of plates (340x16x2.5 mm) was carried out in Ansys – Workbench software on the basis of technical documentation - FIG. 1. The following material properties were set [25]: Young modulus E=200000 MPa, Poisson's ratio υ=0,33.

FIG. 1. Implant for the treatment of chest deformations: a) without modification – Model 1, b) and c) modified with assembly holes – Model 2 and 3

On the basis of the geometrical models a finite element mesh was generated. The meshing was realized with the use of the SOLID187 element.
For the analysis, assumptions were made (FIG. 2):

- in the B and C locations of the bars all degrees of freedom were taken away,
- surface-to-surface contacts interactions between plates, crossbars and locking screws were set up,
- calculations were made for F (A) loading force in 19 steps for loads from 10 N to 100 N every 10 N and from 100 N to 1000 N every 100 N.

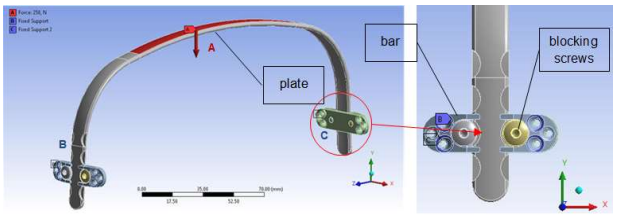

FIG. 2. Presentation of boundary conditions.

Results and Discussion

Obtained characteristics were presented in FIG. 3.

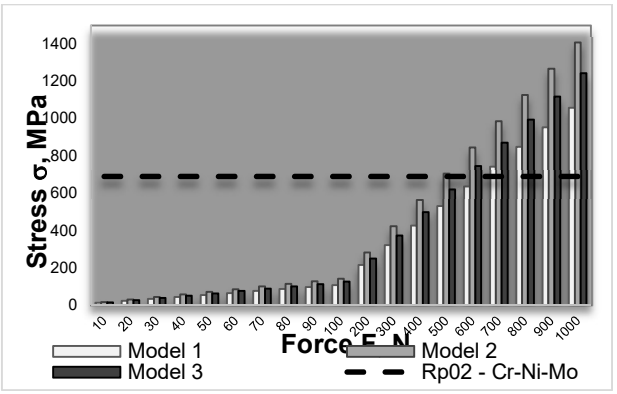

FIG. 3. Characteristics of changes in maximum stresses.

On the basis of the analysis, it can be concluded that the maximum values of the reduced stress occur in the plate in the region of fixing the crossbar. Assuming the maximum loading force F = 1000 N for all analyzed models, these values exceeded the value of the conventional yield point $R_{p0,2}$ = 690 MPa assumed for the selected metal biomaterial. Taking into account this criterion, it can be stated that the maximum safe load for the assumed thickness of the plate was for: Model 1: F = 600 N, Model 3: F = 400 N, Model 5: F = 500 N.

Conclusions

Summing up, it can be concluded that the proposed changes in the geometrical shape of the implant consisting in the addition of openings to facilitate the mounting of locking bars and increasing the possibility of applying the implant to other chest diseases contribute to a slight reduction of the implant's strength in its critical areas. These places constitute the contact area of the panel with the blocking bar.

Acknowledgments

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BIOMIMETIC IN SITU NUCLEATION OF CALCIUM PHOSPHATE/Ag COATING ON BIOINERT CERAMIC

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[ENGINEERING OF BIOMATERIALS 148 (2018) 27]

Introduction

Zirconia ceramics has been commonly used in the dental industry because of its excellent biological, mechanical and aesthetic properties. This material, however, is classified as nearly inert. To bioactivate the ceramic surface, biomimetic deposition of calcium phosphate (CaP) coatings has been established [1]. Another important aspect in the dental field is antimicrobial character of applied materials to minimize or even avoid bacterial infections and tissue inflammation. An addition of Ag nanoparticles could improve an antibacterial character of coated material. Thus, the co-deposition of HA-coating was the focus of the present research. To obtain antibacterial properties of the HA-coating, an additional substance, nanosilver was applied. Nanosilver was co-precipitated simultaneously with the CaP-coating by soaking in the supersaturated simulated body fluid (SBF) solution.

Materials and Methods

2 times concentrated SBF-solution was prepared to precipitate a HAp coating on zirconia surface. Zirconia substrates were pre-coated with CaP-layer by soaking in the SBF-solution for 4 days. Subsequently, three different charges were manufactured. In the first charge, the soaking process in the same SBF solution was continued for next 4 days (1x SBF). In the second charge the solution was refreshed by the new SBF-solution and the samples were soaked in the new solution for further 4 days (2x SBF). In the third charge, the residual solution after 4 days was replaced by a fresh modified SBF-solution, containing silver nanoparticles (2x SBF+Ag) in concentration 0.5%. The reaction of SBF-solution with Ag-nanoparticles was performed for 4 days. Subsequently all samples were removed from the solutions and gently rinsed with purified water.
To characterize the topography of the surface and the chemical composition SEM-EDX-analysis was applied. The phase analysis was conducted by using X-ray diffraction. Moreover, supernatant solutions were investigated according to the chemical composition while depositing by using inductively coupled plasma mass spectrometry (ICP-MS).

Results and Discussion

The successful coating with initial HAp-layer after 4 days soaking in the SBF-solution could be verified by SEM on each SBF-treated sample (results not shown). The coating, deposited by changing the SBF-solution after four days resulted in thicker CaP-layer in comparison to the samples prepared without exchanging the solution and those, containing silver nanoparticles. This effect could be observed on the SEM-images (FIG. 1) and XRD diffraction patterns (FIG. 2.)
The SEM images show significant difference between crystal structures of HAp after different treatments. Without changing the solution (1x SBF) HAp-crystals were much smaller than crystals obtained by refreshing SBF-solution after 4 days (FIG. 1a). The ICP results have shown no changes in the chemical composition of the solution after 3 days of soaking, which indicates that ions resources within the solution after this period were completely exhausted. By exchanging the solution with the fresh SBF, new ions were provided to the system and crystal growth could be continued (FIG. 1b). Interestingly, an addition of Ag-nanoparticle to the solution inhibited a HAp-crystal growth (FIG. 2c). Ag-nanoparticles were incorporated into the solution in their metallic form and additionally a new cubic phase within the CaP-coating was precipitated (FIG. 1c).

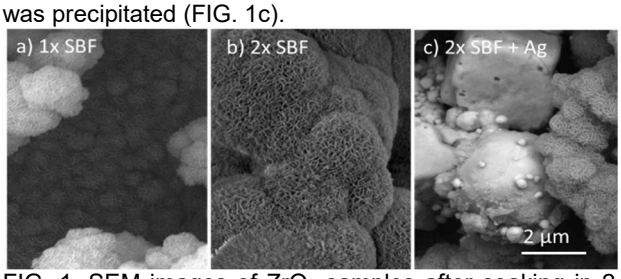

FIG. 1. SEM images of ZrO_2 samples after soaking in 2 times concentrated SBF solution for 8 days (a), for 8 days refreshed after 4 days (b) and for 8 days exchange after 4 days by Ag-containing SBF solution (c).

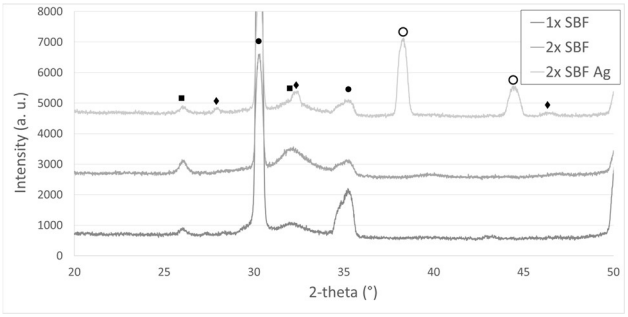

FIG. 2. XRD patterns of HAp deposited coating on zirconia substrates by using deposition in concentrated SBF solution for eight days (lower pattern), for 8 days refreshed after 4 days (middle pattern) and for 8 days exchange after 4 days by Ag-containing SBF solution (upper pattern). Following phases were interpreted: • - ZrO_2, ○ – Ag, ♦ - AgCl, and ▪ - HAp

Conclusions

Coatings obtained by biomimetic approach seem to be promising for implant integration with the surrounding hard tissues compared to coatings obtained using other synthetic methods. Moreover, additional antibacterial nanosilver could be co-deposited to prevent bacterial infections and enhance integration of the implants *in vivo*. Cell culture and bacterial tests are ongoing to evidence a multifunctional character of the new coating.

Acknowledgments

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THE IN VITRO ANALYSIS OF SCAFFOLDS WITH GLYCOSAMINOGLYCANS ADDITION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 28]

Introduction

Glycosaminoglycans are a group of polysaccharides which can be isolated from natural sources such as fish eyeballs or skin [1]. They are non-toxic and biocompatible, which is beneficial for their application in biomaterials science [2]. The use of a natural polymers combination to obtain scaffolds improves their properties such as stability in aqueous environment and mechanical parameters [3].

Materials and Methods

Collagen (Coll) was isolated from rat tail tendons under laboratory conditions. Chitosan (CTS) was purchased from Sigma-Aldrich company (Germany). Glycosaminoglycans (GAGs) were isolated from Salmo salar fish skin (from Koral s. c., Tychy, Poland) with the procedure reported previously [4]. Obtained glycosaminoglycans mixture content was identified by spectrophotometric method. The presence of hyaluronic acid (1.26%) and chondroitin sulfate (2.03%) was calculated based on the standard curves [4].
In the presented study the cytotoxicity test following the cells viability on the experimental materials was carried.

Results and Discussion

Composites (0.5 cm height, 0.12 mm diameter) were soaked in 70% EtOH (water solution) and washed in sterile phosphate buffer solution (PBS; pH = 7.4). For the studies human osteosarcoma cell line SaOS-2 was used. Cells were seeded at the density of 15 × 10^4 cells/composite and cultured for total of 4 days in alpha-MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics. The culture for 4 days was assumed as optimal and sufficient to compare the cells proliferation degree on the composites. Cell-seeded composites were examined with the CellTiter96Aqueous One Solution Cell Proliferation Assay (MTS, Promega, Poland). MTS solution was diluted 10× in phenol-free alpha-MEM and 400 µl aliquots were added per well per sample. The absorbance at 490 nm was measured after 30 min incubation at 37°C in the dark [5].
Results were expressed as% change in cell viability compared to results obtained for unmodified composites. For statistical analysis, the value of P < 0.05 was considered significant.

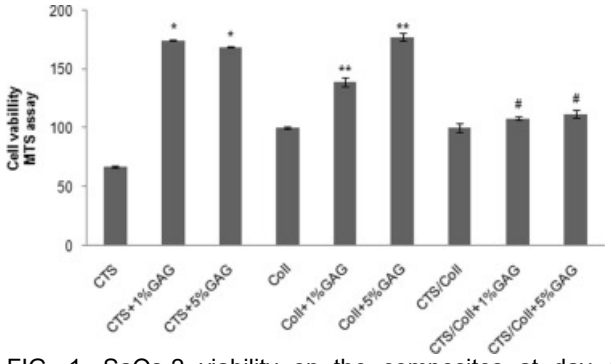

FIG. 1. SaOs-2 viability on the composites at day 4 culture *p < 0.05 vs. CTS; **p < 0.05 vs. Coll; #p < 0.05 vs. CTS/Coll; Student t-test.

The addition of glycosaminoglycans to the composites obtained with the use of chitosan, collagen or their mixture increased cell viability (FIG. 1) and this depended on GAGs amounts. It is plausible that increasing GAGs amounts in polymers led to substantial structural changes of the composites. These changes enhanced the biocompatibility of materials.

Conclusions

Glycosaminoglycans mixture was isolated from fish skin. The obtained porous collagen/chitosan based materials supplemented with GAGs demonstrated higher biocompatibility compared to composites without glycosaminoglycans. The increase of cells viability on composites with GAGs was observed. The study showed that the food industry wastes as fish skin can be used as the natural source of a compound that can be successfully used to prepare biocompatible materials for tissue engineering.

Acknowledgments

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NOVEL ALGINATE AND CHONDROITIN BEADS BASED ON MG AND SI CO-SUBSTITUTED HYDROXYAPATITE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 29]

Introduction

Hydroxyapatite (HA, $Ca_{10}(PO)_4(OH)_2$), due to its similarity to the inorganic component of mineralized tissues is a highly biocompatible, bioactive and osteoconductive material. Therefore, HA plays a crucial role in implantology, dentistry and bone surgery [1,2]. Moreover, ease of ionic substitution allows the introduction of elements having a beneficial effect on bone metabolism – such as magnesium and silicon [3]. Co-substitution of Mg^{2+} and SiO_4^{4-} is believed to stimulate activity and proliferation of osteoblasts, enhance collagen type I synthesis and improve biocompatibility of obtained material [4,5]. Dense HA-based beads, with improved mechanical properties, could serve as bone defect filling material [6]. Future studies will involve the use of granules for local delivery of drug substances to the injured tissue.

Materials and Methods

All samples of pure, as well as substituted HAs, were synthesized via the precipitation method. The obtained samples were physicochemically examined using various analytical methods: mid-infrared spectroscopy (FT-IR), solid-state nuclear magnetic resonance (ssNMR), powder X-ray diffractometry (PXRD) and transmission electron microscopy (TEM). The elemental analysis was conducted by inductively coupled plasma optical emission spectrometry (ICP-OES). The preliminary *in vitro* cytocompatibility was demonstrated on BALB/c 3T3 mice fibroblasts according to ISO guidelines. Then, composite beads based on Mg and Si co-substituted HA, sodium alginate (SA) and chondroitin sulphate (CS) were prepared. Novel alginate-crosslinking reaction based on Mg^{2+} ions as crosslinking agents was used to elevate magnesium concentration in the outer layer of composites.

Results and Discussion

All samples were composed of a single crystalline apatitic phase. As confirmed by TEM imaging, samples were nanocrystalline, with elongated, needle-like crystals. All FR-IR spectra indicated the presence of characteristic bands of HA. All samples turned out completely cytocompatible – there were no detectable zones of growth inhibition or damaged cells. ICP-OES measurements indicated the presence of introduced elements in the amount of approx. 0.25 wt% of Mg and 0.6 wt% of Si, which corresponds to the 85% and below 50% efficiency of Mg and Si substitution respectively. The reduced yield of orthosilicate ions substitution is probably due to the competition with carbonate ions, derived from the air and the reagents. Multicomponent granules, composed of MgSi-HA, SA and CS were successfully obtained. Crosslinking of Mg^{2+} ions allowed the introduction of additional 2.6 wt% of magnesium in the composite beads. The obtained material is characterised by low porosity, which is probably caused by the addition of CS. Thus, we anticipate a gradual release profile, which will be examined in the next stage of our research.

Conclusions

The current research concerned the synthesis and subsequent physicochemical characterization of HA doped with Mg^{2+} and SiO_4^{4-} ions, including their co-substitution. Single-phase, nanocrystalline and cytocompatible HAs with approx. 0.25 wt% of Mg and 0.6 wt% of Si were obtained. Novel hybrid composite beads were prepared, which could potentially be used for local delivery of ions and drugs.

Acknowledgments

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EFFECT OF VASCULAR SCAFFOLD COMPOSITION ON RELEASE OF SIROLIMUS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 30]

Introduction

Bioresorbable vascular scaffolds (BRS) have been designed to provide mechanical support against acute recoil to treat arterial restenosis and to overcome the complications of metallic drug-eluting stents (DES) e.g. vascular inflammation, hypersensitivity reactions and incidence of thrombosis [1,2]. Many studies have shown that the side effects of DES and BRS still remain, e.g. inflammation, late thrombosis, and late restenosis. The lack of capacity for adjusting the drug dose and inadequate release behavior are one of the main reasons of these side effects [3]. Therefore, the drug release rate has become one of the important criteria for the evaluation of drug-eluting stents. The objective of this study was to develop degradable sirolimus-eluting polymer coatings applicable to bioresorbable polymer-based scaffolds. Moreover, a detailed analysis of sirolimus release and degradation of scaffolds has been conducted. So far, mainly polymeric coatings of metallic stents have been studied in regard to explain drug release mechanisms.

Materials and Methods

Two kinds of scaffolds models obtained by microinjection molding from poly(lactide-co-glycolide-co-trimethylene carbonate) (length 16 mm; Ø 6 mm) were coated by dip coating method with sirolimus eluting layer composed of poly(L-lactide-co-trimethylene carbonate) (poly(L-lactide-co-TMC); PLLA/TMC). *In vitro* degradation and drug release study was conducted for 180 days at 37°C in modified sodium chloride (0.9%). Scaffolds explanted from pigs were used for evaluation of *in vivo* degradation and drug release. Quantification of sirolimus was performed at the wavelength of 287 nm using a high performance liquid chromatography (HPLC). The BRS were characterized before and after degradation. Changes in the polymer composition were monitored on the basis of ^{1}H NMR spectroscopy. The molar mass and molar mass distribution of the polymer were determined by gel permeation chromatography (GPC). Morphology of scaffolds was observed by means of polarizing microscopy and scanning electron microscopy (SEM).

Results and Discussion

Two kinds of scaffolds obtained from poly(lactide-co-glycolide-co-TMC) with lower and higher lactide content (scaffold 1 and scaffold 2, respectively) were coated with solution composed of poly(lactide-co-TMC) and sirolimus. The surface of scaffolds with drug-eluting layer of ≈ 2.7 µm thickness (FIG. 1C) was smoother than uncoated scaffold (FIG. 1A), but had numerous, tiny cavities (FIG. 1B).

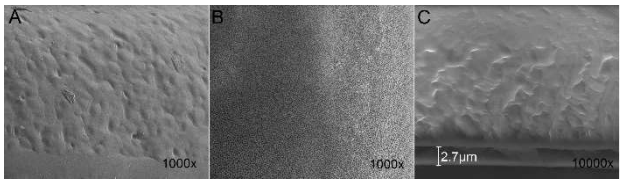

FIG. 1. SEM images of the surface of scaffold 1: uncoated (A), coated (B) and coated cross-section (C).

The developed coatings showed controlled release of antiproliferative agent with elimination of burst effect (FIG. 2). However, differences in degradation and drug release profile from two kinds of scaffolds were observed. Scaffold 2 composed of polymer with higher lactide content showed slower and bi-phasic, erosion-controlled release of sirolimus. On the contrary, sirolimus release from scaffold 1 composed of polymer with lower content of lactide was mainly controlled by diffusion.

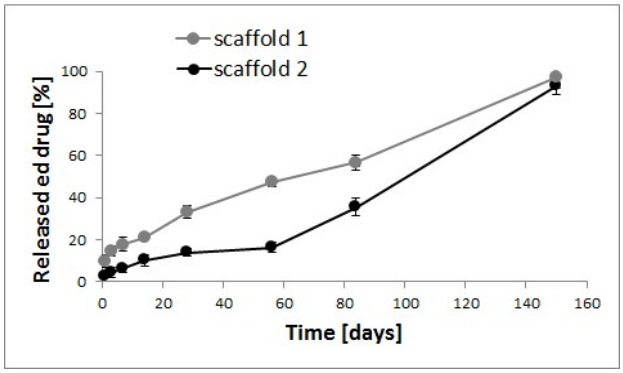

FIG. 2. In vitro release of sirolimus from scaffolds (S.D. shown as error bars, n = 3).

Conclusions

The biodegradable coatings of vascular scaffolds providing regular release of sirolimus were developed. The study demonstrated that characteristics of scaffold and its degradation is a crucial factor that must be considered in development of bioresorbable vascular scaffolds with controlled release of antiproliferative agent.

Acknowledgments

This study was conducted in the frame of project of The National Centre for Research and Development No. PBS3/A9/38/2015 "Obtaining of self-expanding, polymeric and biodegradable, drug-eluting vascular stents".

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EVALUATION OF VARIOUS ANTIBACTERIAL, BIODEGRADABLE POLYMERIC COATINGS DEVELOPED ON Ti6Al7Nb SURFACE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 31]

Introduction

Different reports on the use of ciprofloxacin in case of severe bacterial infections can be found in the literature [1,2]. It is a broad-spectrum antiinfective agent of the fluoroquinolone class. Ciprofloxacin has *in vitro* activity against a wide range of gram-negative and gram-positive microorganisms. The mechanism of action of quinolones, including ciprofloxacin is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin [5].

Aliphatic polyesters and polyestercarbonates are frequently used as a drug delivery systems for controlled release of different active substances like: antibiotics, growth factors, and hormones [3-6]. Ciprofloxacin has also been incorporated into drug delivery systems which based on polyesters, like: poly(lactide-co-ε-caprolactone). From the other hand, Titanium (Ti) is widely used as a biomedical material since it has extraordinary mechanical properties, high corrosion resistance and satisfactory inherent osseointegration ability [7]. It is used in orthopedic and dental applications. Its biomedical applications are connected with their good biocompatibility and corrosion resistance [8].

The objective of presented study was the comparison of wide range of antibacterial bioresorbable polymeric coatings developed on titanium-based prototype forms of the implants. The therapeutic function of presented metal/polymer+drug systems was confirmed.

Materials and Methods

Various kinds of polymers like:
1.Poly(glycolide-ε-caprolactone) (10/90) **P(G/Cap)**
2.Poly(glycolide-ε-caprolactone-L,L-lactide) (10/12/78) **P(G/Cap/L)**
3.Poly(L,L-lactide-trimethylenecarbonate)(74/26) **P(L/TMC)**
4.Poly(lactide-trimethylenecarbonate-glycolide) (10/12/78) **P(L/TMC/G)**
5. Poly(D,L-lactide-glycolide) (84/16) **P(LG)**
were used to prepare coatings on the metallic samples (rods). Polymers were synthesized in bulk by the ring opening polymerization (ROP) using Zirconium (IV) acetylacetonate $Zr(acac)_4$ (Aldrich) as a non-toxic initiator. In the next step, polymers solutions (1%w/w, solvent: CH_2Cl_2) have been used to coat the metallic samples by dipping method (Dip Coater, MTI Corporation, 1,2,3 layers, 30 s of immersion time). Coated rods were characterized according to the following techniques: *^{1}H- and ^{13}C-NMR spectroscopy (*600 MHz Bruker Avance II Ultrashield Plus spectrometer), *Gel Permeation Chromatography* GPC (Physics SP 8800 chromatograph and detector: Shodex SE 61), *Optical Profilometer* (Sensofar).

Antibacterial activity of ciprofloxacin has been examined with the use of the Escherichia coli (E. coli; ATCC® 25922™). Coated titanium-based implants were placed in tubes with 10 mL of Bacterial suspension ($150x10^6$c.f.u./ml) in Trypcase Soy Broth (TSB)) and in tubes incubated at 37°C. Uncoated implants and TSB medium without bacteria was used as a control.

The bacterial growth on the media was studied at 24, 48, 56 and 72 h. The average number of bacteria was measured by Trypcase Soy Agar plate count. The experiments were independently done in triplicate and means and standard deviations were calculated.

Results and Discussion

In the study we present detailed analysis of the various kinds of polymeric materials based on polyesters and polestercarbonates as antibacterial coatings for Ti6Al4V implants intended for short-term therapy. The aim of using biodegradable polymers was to introduce therapeutic functions of biomedical implants. We obtained this by incorporation of the ciprofloxacin molecules into the polymeric matrix. Intended function has been confirmed. It appeared that all kinds of ciprofloxacin enriched polymeric coatings show bactericidal properties. Five different ciprofloxacin loaded polymeric coatings have been incubated in bacterial suspension. The bactericidal effect was observed after 24 h in the case of P(LG)+CFX, after 48 h in case of P(G/Cap/L)+CFX and after 72 h of exposure to P(G/Cap)+CFX and P(L/TMC/G)+CFX. The slowest bactericidal effect was observed for P(L/TMC) (96 h).

Additionally, it was analyzed that:
i) the type of material e.g. copolymer composition and
ii) the numbers of dipping influence on: release of the ciprofloxacin as well as on the coatings thickness.

Conclusions

Various kinds of biodegradable polymeric coatings formed on Ti6Al4V alloy were successfully developed. The antibacterial function of presented coatings has been confirmed.

Acknowledgments

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OPTIMIZATION OF MICRO-INJECTION MOULDING PROCESS STABILITY IN AUTOMATIC-CYCLIC MODE CONDITIONS DURING MANUFACTURING BIORESORBABLE VASCULAR STENTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 32]

Introduction

Processing of biodegradable polyesters creates many difficulties due to their susceptibility to hydrolysis, especially at high temperatures and fairly poor thermal stability [1,2]. For this reason, they are quite rarely used in industry. However, thanks to their unique properties, they have found quite a wide application in medicine, e.g. for the obtaining of vascular implants – stents [3]. Production of this kind of implants, poses a special challenge due to their thin-walled, openwork structure.

Due to the relatively low energy and polymer material consumption, as well as the process rate, micro-injection moulding seems to be the best method for obtaining bioresorbable stents. As a result of carrying out the process in high temperature and generation of the high internal friction caused by injection under high pressure of molten polymer to a very small space of the injection mould cavity (wall thickness of the element 200 μm), the polyester material is exposed to thermal degradation. It causes weakening of the internal structure of the stent, which prevents its connection to the insertion system, due to span cracking.

The present work focuses on optimizing the micro-injection moulding process of vascular stents, made of poly(L-lactide-co- glycolide-co- trimethylene carbonate), in terms of maintaining mechanical properties in processing carried out in an automatic cyclic mode.

Materials and Methods

The study was based on a terpolyester poly(lactide - *co*-glycolide-*co*-trimethylene-*co*-carbonate) with a mutual molar ratio of comonomers 76 : 15 : 9 respectively [4], prepared in the form of a pellet of the dimensions 2 mm by 2 mm.

The polymeric material was processed using a MicroPower 15 micro-injection moulding machine (Wittmann Battenfeld). The mould was thermostatted with a hot water. The moisture content of the granulate was measured by Karl-Fischer titration (Metrohm). The quality of stents and regions susceptible to damage was observed with a stereoscopic microscope (DeltaOptical) working in polarized light. The mechanical properties of the implants were measured on the crimper device (tensile test machine from Blockwise Engineering) intended for measuring radial forces [RF]. The progress of thermal degradation was estimated based on changes in average molecular weights obtained by gel permeation chromatography (GPC). The influence of processing parameters on the thermal properties of stents has been examined by differential scanning calorimetry DSC.

Results and Discussion

The obtained results indicate, that the maximum moisture content in the used terpolymer, allowing to limit the hydrolysis under processing conditions (200°C and 10 min of contact time) is 250 ppm. Optimal stent geometry (FIG. 1), with minimization of degradation, was obtained by using the parameters listed in TABLE 1.

TABLE 1. Micro-injection molding parameter process.

Injection limiting pressure	2500 bar
Volumetric melt stream	4 ml/s
Holding pressure	1500 bar; 5 s
Mold cavity temperature	55 °C
Conditioning in closed mould	15 s
Injection volume	0,25 ml

The graph below (FIG. 2) shows the correlation between the radial force (RF) of the stent and the average molecular weight (Mn) in subsequent processing cycles (60 s per cycle). An average radial force value of 27.2 ± 1.5 N was obtained, with an average molar mass equal to 29.2 ± 2.2 kDa (initial molar mass of 38 kDa). Small standard deviations indicate good stability of the terpolymer micro-injection process during 13 cycles. After this time, the plasticizing system of the injection molding machine should be reloaded with a fresh portion of polymer.

Conclusions

It has been proven, that the selection of parameters allowing the cyclic production of biodegradable vascular stents with the desired utility properties is possible by micro-injection molding method. This is crucial for transferring the production scale from laboratory to industry.

Acknowledgments

This study was conducted in the frame of project of National Centre for Research and Development No. PBS3/A9/38/2015 "Obtaining of self-expanding, polymeric and biodegradable, drug-eluting vascular stents".

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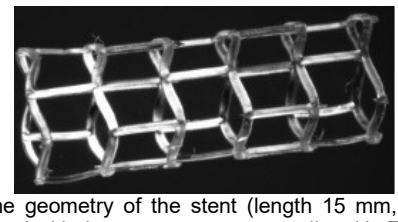

FIG. 1. The geometry of the stent (length 15 mm, diameter 6 mm) obtained with the process parameters listed in TABLE 1.

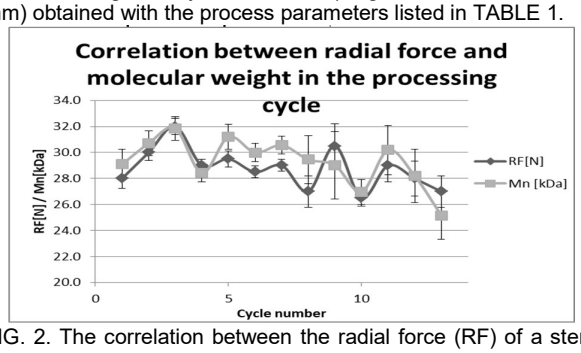

FIG. 2. The correlation between the radial force (RF) of a stent and the average molecular weight (Mn) in subsequent processing cycles (60 s per cycle).

DRUG - ELUTING BIORESORBABLE VASCULAR STENTS MANUFACTURED BY MICROINJECTION MOULDING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 33]

Introduction

Since FDA approval for peripheral vascular in 1990 and for coronary vascular procedures in 1994, tens of millions of people in the world have undergone a coronary or peripheral stenting procedure. Today, because of frequently encountered late complications related to implantation even the newest generation of metal vascular stents, optimal treatment of coronary heart and peripheral artery disease entails the need to search for new solutions. Looks that stents manufactured with use of biodegradable polymeric materials represent an attractive alternative, which allow revascularization modality and finally full regeneration of the vessels [1]. The most commonly used polymers for bioresorbable stents are poly(L-lactide) (PLLA) and poly(lactide-co-glycolide) (PLGA) [2]. However, the future of such stents is not obvious, as evidenced by the withdrawal from the sale of previously approved and commonly used Absorb stents (Abbot Vascular). However, this does not mean, that this company and others are giving up for research on this technology [3]. The selection of optimal material, implantation techniques and production technologies are still objects of intensive investigations. Now, there are at least 15 bioresorbable stent programs in progress, with five in the advanced development stages.

Two years ago, in our previous performance [4], we presented the preliminary results of our work on obtaining biodegradable vascular stents based on L-lactide/glycolide/ trimethylene carbonate terpolymer using the new technology of microinjection moulding.

Today, in the study we present more advanced results showing possibility of manufacturing on this way peripheral and coronary stents, their properties and tests of surgical suitability carried out in vivo on a domestic swine model.

Materials and Methods

The L-lactide/glycolide/trimethylene carbonate terpolymer was obtained in bulk by two-step synthesis. In the first step, TMC oligomer was prepared by ROP using zinc acetylacetonate as a catalyst and butandiol as initiator. The obtained oligocarbonate was used in the second stage of the synthesis as a macroinitiator of the copolymerization reaction of L-lactide with glycolide. Obtained product was granulated and used in further processing. In the production of stents, MicroPower 15t micro-injection moulding machine was used with injection moulds made in the Alexander TOOLS (Chwaszczyno) company. The stents mouldings were covered with a polymer layer containing sirolimus, then two tags from platinum were affixed to the end stent segments. The finally stents were conditioned at 60°C to stress relaxation, final crystallization and elimination of post-processing contraction. The stents were crimping on the balloon dilatation catheters Mozec (Meril) with using specialist crimping machine at 45°C. After packaging, the produced implantation systems were sterilized with electrons beam at a dose of 15kGy. A total of 25 stents were implanted into the peripheral arteries (profunda femoris) of 10 animals. All stents were implanted under quantitative coronary angiography (QCA) guidance. Independently during procedures performed on animals was assessed the possibility and behavior of stents during conducted treatment as well as course of implantation using the techniques of intravascular imaging OCT (Optical Coherent Tomography).

Results and Discussion

The investigations were conducted with terpolymers of different composition (70% L-LA 10% GL 20% TMC, 82% L-LA 10% GL 8% TMC, 76% L-LA 9% GL 15% TMC), which were synthesized within the framework of the project. All of them, proved to be more suitable for injection processing compared to the previously used commercial poly (L-lactide). Using synthesised terpolymers, a better filling of the mould nest was obtained, mainly due to the high melt flow rate at the processing temperature (relatively small molecular weight terpolymers of about 40-50 kDa, and possess microblock chain microstructure). The optimization of the processing conditions and of heating and cooling of injection moulds allowed for obtaining stents with properties very close to the assumptions. Manufactured stents were subjected to mechanical tests and attempts of optimization of the crimping process on the catheter and opening at conditions occurring during surgery. A series of manufactured implantation systems with peripheral stents have been used in the preclinical studies on the swine model. The first results of these studies are rather promising, but unfortunately they presented the lack of the required repeatability of the properties of the produced stents, which was manifested by their collapsing few days after implantation. On the other hand, it was shown that a part of the examined stents maintained the vascular walls properly and displayed the self-expanding effect after the implantation.

Conclusions

It has been confirmed that by adapting a suitable polymeric material with a relatively low viscosity in the processing conditions and good mechanical properties, having the appropriate shape of the final stent and the precise injection machine allowing rapid injection under high pressure, it is possible to obtain such thin-walled products as vascular stents. It seems that by further process optimization, and mainly achieving repeatability of all operations, the stents with good functional properties possible to use in clinical treatments can be obtained.

Acknowledgments

This study was conducted in the frame of project of National Centre for Research and Development No. PBS3/A9/38/2015 "Obtaining of self-expanding, polymeric and biodegradable, drug-eluting vascular stents".

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ELECTROSPUN, BIODEGRADABLE, NON-WOVEN DRESSING WITH ADDITION OF PROPOLIS FOR DIFFICULT-TO-HEAL WOUND TREATMENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 34]

Introduction

The selection of the optimal dressing is crucial for the wound healing process. Traditional dressings including gauze, adhesive tapes and bandages are used to protect against contamination and mechanical damage of the wound. However, modern dressings are designed not only to cover the surface of the wound, but also to interfere with the complex healing process, accelerate it and minimize complications. Furthermore it should provide a moist wound environment, accelerate reepithelialisation, accelerate angiogenesis and synthesis of connective tissue, allow gas exchange between the wound and the environment, provide optimal wound temperature to increase blood flow within it, pose barrier to infection, do not adhere to the wound , minimize unpleasant smell, support the migration of leukocytes and enzymes, be transparent, allow to observe the healing process, be sterile, non-toxic and non-allergic [1-3].

A very interesting alternative for simple dressings are complex dressings containing a polymer with an incorporated active compound. As a result, not only the optimal wound healing parameters related to the influence of the active compound can be obtained, but advanced manufacturing systems, like electrospinning process, allow to control the pharmacokinetic processes in such a way as to achieve a prolonged action of the drug within the healing wound [1-3].

Propolis is a natural resin and wax substance produced by honey bees as a repair and protective material. This substance has proven activities such as: antioxidant, anti-inflammatory immunomodulatory, antiviral, antineoplastic, antibacterial, antifungal, stimulates reepithelialisation and shortens wound healing time [4].

The aim of this study was to obtain a wound dressing from a biodegradable polymer containing propolis, for the treatment of difficult-to-heal wounds, which releases propolis in a controlled manner throughout the treatment and creates favorable conditions for the regeneration of damaged cells, while ensuring a sterile wound environment, and after the end of treatment, it degrades, which allows you to avoid discomfort associated with the removal of the dressing.

Materials and Methods

Wound dressings has been obtained by electrospinning from a poly(lactide-co-glycolide) copolymer solution (mutual molar ratio of comonomers: 85% lactidyl units to 15% glycolidyl units. Propolis content: 5 wt% and 10 wt% relative to the polymer. Solvent: 1,1,1,3,3,3-hexafluoro-2-propanol. Potential difference: 27kV. Distance between the electrodes: 15cm. Solution dose rate: 1.5 ml/h, volume: 22ml [2].

The *in vitro* drug release conditions: shaking in Phosphate Buffered Saline (PBS), in an incubator at 37ºC, through 40 days. Propolis amount was measured via HPLC method.

For *in vivo* research, two White Ursus pigs, were used. The 21-day experiment consisted in observation of healing 3º burn wounds supplied with tested mats. Research was made according to the standard Hoekstra model. 4 research groups was set: 2 study groups: wounds supplied with 5 wt% of propolis mats, wounds supplied with 10 wt% of propolis mats and 2 control grups: wounds supplied with mats without propolis and wounds supplied with NaCl twice a day. Healing processes were compared basing on the macroscopic changes.

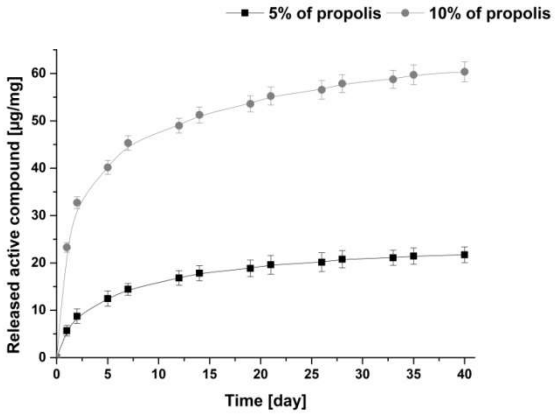

FIG. 1. Release of propolis from PLGA 85/15 electrospun wound dressings. Results are presented as amount of released propolis [µg] per 1 mg of polymer mat (MEAN±SD, N=3).

Results and Discussion

FIG. 1 presents release of propolis from electrospun polymer mats. After initial intensive release (which is beneficial for saturation with the active compound), the further release proceeded evenly. The experiment showed the most beneficial effect of dressings containing 5% addition of propolis, then dressing dressings with 10% addition, in relation to dressings without added propolis and wounds healing with “the forces of nature”. Furthermore, the applied dressings caused a better therapeutic effect than the usual 5% propolis ointment or the silver sulfadiazine salt used usually in the treatment of burns (external control groups from previous studies).

Conclusions

Biodegradable, non-woven dressings with the addition of propolis allow the release of the apitherapeutic in a controlled manner, allowing the patient to avoid discomfort associated with the change of dressing or daily local administration of the drug substance, at the same time giving a better therapeutic and aesthetic effect, which makes them a potentially beneficial solution for treating difficult-to-heal wounds for the future.

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THE ELECTROSPUN FABRICS AS A HEART VALVE LEAFS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 35]

Introduction

The population at risk for heart disease especially aortic stenosis is increasing. The estimation of this risk is 80% of adult population in which may appear symptoms of aortic stenosis. The survival rate for patients who begin experiencing its symptoms is 50% at 2 years and 20 % at 5 years without aortic valve replacement.
There are two ways of intervention in this case: surgical (open chest) and less invasive transcatheter aortic valve implantation (TAVI) also called TAVR, transcatheter aortic valve replacement. The TAVI procedure is applied mostly for patient for whom an open heart procedure poses intermediate risk. For that reason patients in their 70s or 80s are better candidates for this type of surgery.
The TAVI procedure is surgical minimally invasive, repair the valve without removing the damaged valve. Instead of old valve the new is placed into the aortic valve's place.
The TAVI heart valve construction is based on metal stent with leafs attached which is before implantation crimped to the low dimeter on catheter balloon. The crimped form is entered through the femoral artery (large artery in the groin) which does not require a surgical incision in the chest or entering through a large artery in the chest or through the tip of the left ventricle (the apex), which is known as the transapical approach.
[1] Herein, it is described the results of mechanical test investigation on non-woven matts obtained *via* electrospinning and matts obtained *via* combined methods (electrospinning – electrospray). The tested fabrics were also easily assembled on stent. The preliminary result of mechanical test of formed heart valve leafs are presented.

Materials and Methods

The synthetic polymer for medical use was: Chronoflex Ar 22%(polyurethane-co-carbonate)(PU) manufactured by AdvanSource. The polymer was dissolved in DMAc to concentration 8% (electrospray purpose) and 18% (electrospinning purpose). The collector of electrospinning unit was modified for the valve leafs production and stent assembling occurring simultaneously. The parameters of electrospinning technique were optimized for electrospray film deposition and electrospinning fibers formation.
The mechanical test of material was determined using 4204 (Instron, Norwood, USA), crosshead section rate was 20 mm/min.
The mechanical test of heart valve leafs was determined using pump VDT-3600i(BDC Labs)

Results and Discussion

The electrospun fabric is characterized with lower value of stress and strain at the break in comparison to the film but fabrics may easily change its shape (not-merged fibers poses ability to move, presence of free volume-porous). Therefore more than 70% of multilayer fabrics thickness is built with fibers (see table combined sample). The rest (30%) of sample thickness is mix or film only. This mixed type of construction allows to seal the porous fibers material and prevent the blood seeps through the leafs.

TABLE 1. The results of mechanical tensile test of various form of samples

Lp.	samples (d=150μm)	Stress at the break [MPa]	Strain at the break [%]
1.	fibers	17.77±5.38	475.40±47.66
2.	film	27.38±3.71	884.25±15.20
3.	Combined (film and fibers)	32.47±2.24	745.80±44.26

Additionally, during stent crimping, attached material (combined fiber and film) stays in continues phase without any perforation. Macroscopic observation of heart valve after 60 days pump test (accelerated fatigue test simulation of 360 days heart valve work) revealed positive condition of all heart valve joint, although the material increased its dimension (stretched *ca.*10%). The stretch effect appeared during two starting days of fatigue test. Probably, fibers are being oriented under flow pressure in favorable shape.

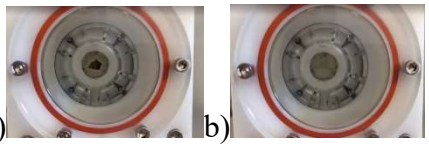

FIG. 1. Mechanical test of heart valve leafs: a) leafs position-open, b) leafs position-closed [2]

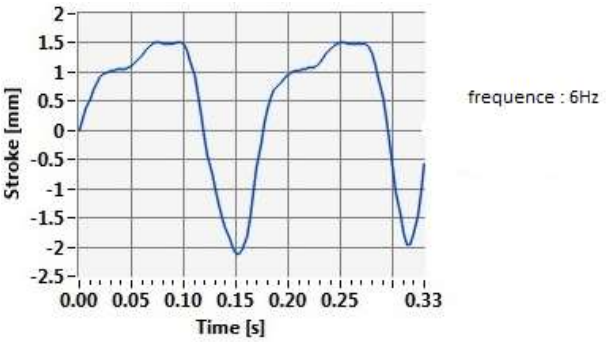

FIG. 2. The shape of wave during mechanical test of heart valve

Conclusions

The electrospun multilayer PU product is promising for the heart valve leafs forming. The mixed form can be easily attached to metal stent during electrospinning processing which decreases the time of the heart valve production significantly (lower end-cost). Nowadays, the sewing heart valve process takes more than 2 days of handcraft work. Additionally, the appearing deformation may help to obtain favorable shape of leafs.
Further work will be focused on production of valve possessing stable shape during fatigue tests.

Acknowledgments

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DESIGN AND MANUFACTURE OF CUSTOMIZED MEDICAL IMPLANTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 36]

Introduction

In the years 2006-2010 research on the subject of custom medical implants was carried out at the Department of Biophysics, Institute of Materials Science, Lodz University of Technology. The aim of this work was to assess the possible advantages of custom implants and whether their use was clinically justifiable. The study was carried out as a PhD programme and resulted in the clinical application of custom orbital implants. The results of this research were later commercialized in the form of a newly created Custom Medical Implants Unit that was established in 2011 at Bionanopark Ltd. To date, as a result of this research, over one hundred custom maxilla-facial and cranial implants have been designed, produced and clinically implemented (orbital wall reconstruction, cranioplasty, mandibular reconstruction). Each year, over 20,000 leg amputations are carried out in Poland as a consequence of different clinical conditions. These patients later require a limb prosthesis and physiotherapy in order to regain mobility. The standard prosthetic device that is commonly used to treat such patients, is the socket-suspension type prosthesis system, which unfortunately transfers loads through the soft tissues of the limb stump. However, there is an alternative method of treatment, which involves the use of osseointegrated implants that facilitate direct skeletal attachment of a prosthesis [1,2]. On the basis of the available data, we recognized a significant need for custom designed, osseointegrated percutaneous orthopaedic implants, which at present are not generally available. In our opinion the design and manufacture of such prostheses is worthwhile and should be developed into a clinically viable medical device.

Materials and Methods

The design stage must take into consideration such aspects as conforming these bespoke implants to individual and unique patient anatomy, implant mechanical strength analysis and its integration with bone tissue and selection of an appropriate biomaterial as well as a structural analysis of its surface. On the other hand, the manufacturing process must take into account additive techniques (3D printing), subtractive methods (CNC milling) and hybrid technologies, which can be used to make precise, controlled implant surface modifications. In addition to this, validation of different sterilization methods for such products as well as post-sterilization structural analysis, biocompatibility and thrombocompatiblity must be evaluated.

Results and Discussion

The above concept of designing and manufacturing osseointegrated percutaneous implants formed the basis of a project that was prepared by the authors and subsequently positively evaluated in the POIR programme 1/4.1.4/2017 and accepted for funding by the National Center for Research and Development.
The project consists of the following seven stages - implant design and biomaterial selection, implant mechanical strength assessment and integration with bone tissue (both theoretical and experimental approach), implant manufacture (including surface modification), validation of implant sterilization techniques and post sterilization biological evaluation (biocompatibility, thrombocompatibility and induction of neoplasia or tumor recurrence) and most importantly implant clinical application. The project will be implemented by four units within Lodz University of Technology (the project leader), Bionanopark Ltd, PAFANA SA and Medical University of Lodz and will last for 36 months starting from June 2018.

Conclusions

Finally, the developed methodology for designing and manufacturing such custom orthopaedic implants, will be implemented and commercialized by Bionanopark Ltd. In the initial phase implants will be available for clinical applications in Poland and later on foreign markets, also.
In the following years we will inform you about the progress of our work and implementation of the project.

Acknowledgments

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MODIFICATION OF HYDROXYAPATITE AS A FILLER FOR PMMA BONE CEMENTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 37]

Introduction

Bone cement is a popular biomaterial used in medicine for the restoration of pathological loss of bone and the fixation of prosthesis. Its properties must meet strictly defined criteria that determine the success of the therapy, health and even life. Although, there are many types of bone cement already designed and widely used. This study focuses on a particular type of bone cement – polymethylmethacrylate (PMMA) bone cement. It is extinguished by high mechanical strength, but contains a toxic, carcinogenic residual monomer (about 4-7%), has a low adhesion to bone cells and its polymerization results in both: exothermic effect and shrinkage. Publications in the recent literature indicate that additive of natural component like hydroxyapatite (HAp) may have a positive impact on these features [1,2]. Even the fillers with highly valuable properties cannot fully perform its role when there is a problem with its dispersion in polymeric matrix. Small size of filler's particles determines tendency to creation of aggregates and agglomerates. It worsens dispersion and results in lack of homogeneity in composite's properties. Problem could be solved by prevention of agglomerates' creation. It is possible due to the modification of nanofillers [3,4]

Materials and Methods

Hydroxapatite was made by a wet method and its surface was modified by using the RF PECVD (*Radio Frequency Plasma Enhanced Chemical Vapour Deposition*) technique. Modified HAp was examined regarding to the wettability of the surface (tensiometric method) and chemical structure by FTIR measurements (*Fourier Transform Infrared Spectroscopy*). Finally, it was mixed with the polymer in order to create bone composite. Surface morphology of the bone composite was evaluated using Scanning Electron Microscopy (SEM) and chemical structure with FTIR. Thermodynamic characteristic was obtained after DSC (*Differential Scanning Calorymetry*) examination. Evaluation of the mechanical properties was possible due to the hardness measurements in Shore D scale. Wettability of the composite was established on the basis of the drop shape analysis (DSA) method.

Results and Discussion

Optimization of plasma-chemical modification was made due to Fourier Transform Infrared Spectroscopy. Chemical structure of HAp was frequently measured in order to check in which conditions (glow discharge power, gas flow) the surface is modified on the biggest extend. It turned out that not always the highest values of the power and flow of the methane were required to obtain detectable changes in chemical structure. Methyl groups could be observed on HAp's surface. Measurements of the surface free energy proved that powder after modification is more hydrophobic. It allows to make the surface of HAp similar to the organic matrix (PMMA). This property is especially valued when it comes to prevention of aggregates' creation. Obtained bone medium was measured regarding to its wettability. Its surface turned out to be hydrophobic. Plasma-chemical modification of HAp and improved technique of mixing it with bone cement results in homogenic structure of the composite. Hydrophobic surface of hydroxyapatite prevents creation of its agglomerates. The incorporation of modified hydroxyapatite slightly decreases hardness of the composite, and it is a desirable effect. With increased concentration of hydroxyapatite composites become less brittle. Differential Scanning Calorymetry helped to prove that higher concentration of HAp in PMMA bone cement shortened a little bit the polymerization process. Incorporation of hydroxyapatite minimize generation of the heat.

Conclusions

Modified HAp indicate hydrophobicity and its surface free energy decreases. The morphology of the composite is more homogenic when HAp is modified. The drop of hardness is slightly higher for modified filler in comparison to nonmodified one. Time of polymerization is shorter when the composite includes higher concentration of HAp.

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BIOCOMPATIBILITY OF OXIDIZED ANODICALLY TITANIUM ALLOYS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 38]

Introduction

The most commonly used procedure for modification of surface layer of titanium alloys is anodic oxidation improving biocompatibility [1,2]. Physical and chemical properties of the produced layers depend not only of the parameters of the anodizing process, but also on the preliminary, preceding mechanical and electrochemical surface treatments [3,4]. Biocompatibility studies, in particular the cytotoxicity of anodically oxidized titanium alloys do not include the concentration of metal ions in the extract obtained from the tested samples.
The study determined the effect of extracts from titanium alloys with a modified top layer on the survival of SaO2-2 cells. In addition, tests of the concentration of metal ions in the applied extracts were carried out.

Materials and Methods

The research were carried out for surface layers produced on samples from rods of Ti6Al4V and Ti6Al7Nb alloys. The surface modifications of the samples included pre-treatment treatments: grinding (1), vibratory processing (2), mechanical polishing (3), sand blasting (4), electrolytic polishing (5), anodic oxidation (97V) (6) and steam sterilization (S) [X]. Cytotoxicity tests were performed in accordance with PN-EN ISO 10993-5 and PN-EN ISO 10993-12. Samples with a modified top layer were used on Ti6Al4V ELI and Ti6Al7Nb alloys substrates. Tests were carried put using the model cell line SaOS-2 (bone cancer, osteosarcoma, *Human Osteosarcoma cell line*, CLS, Cat. No. 300331). The MTS test was carried out, which involved determining the amount of formazan in the cells that only living cells are able to produce. As a positive control, sodium dodecyl sulfate was used, the negative control was a culture medium with fetal bovine serum. Cell survival in the test, positive and negative control groups was determined by measuring the amount of formazan formed during 72-hour cell incubation with titanium alloy extracts, sodium dodecyl suflate and fetal bovine serum. The amount of formazan was determined in absorbance studies at 490nm. In addition, microscopic evaluation of the effect of extracts on the SaOS-2 cell line was carried out. Immunofluorescence staining was used to visualize the cytoskeleteon of cells at the microtubule level by observing tubulin protein. Hoechst dye was used for imaging the nucleus. The concentration of metal ions in the extracts of titanium alloys with a modified surface layer used in cytotoxicicty and microscopic observations of cells was determined by the ICP-AED method.

Results and Discussion

In the cytotoxicity tests, the slightly different degree of interaction of their extracts with the survival of SaOS-2 cells was found. The criterion for assessment – PN EN ISO 10993-5 standard – allows to state that the produced layers and their degradation products do not cause cytotoxic effects. Microscopic examinations of SaOS-2 cells in both control and incubated with Ti6Al4V ELI and Ti6Al7Nb alloys extracts with a modified top layer showed no change in morphology and cytoskeleton organization (FIG. 1). The cells adhered evenly to the substrate and were properly flattened. At the same time lamellipodia/filopodia testify to proper migration.
Cells were also observed in the division phase confirming proliferation. Analysis of the chemical composition of titanium alloy extracts with a modified surface layer indicates slight differences in the concentration of elements (Tab. 1). High concentrations of vanadium ions in extracts of Ti6Al4V, niobium ions in Ti6Al7Nb alloy extracts and low concentration of aluminum ions for both alloys should be emphasized.

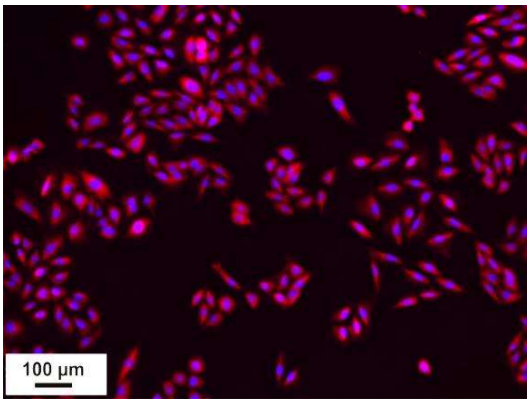

FIG. 1. Image of cells subjected to incubation with extracts from Ti6Al4V alloy of modified surface 1/3/4/97V/S

TABLE 1. Concentration of metal ions in Ti6Al4V and Ti6Al7Nb alloy extracts with modified Surface layer

Alloy	Modification method of surface	Concentration of metal ions, ppm							
		Ti	σ_{SD}	Al	σ_{SD}	V	σ_{SD}	Nb	σ_{SD}
Ti6Al4V	1/2/3/4/97V/S	1.05	0.09	0.65	0.10	0.91	0.05	–	–
	1/3/4/97V/S	1.10	0.10	0.65	0.10	0.80	0.03	–	–
	1/3/4/5/97V/S	1.20	0.10	0.67	0.10	0.81	0.03	–	–
	1/2/5/97V/S	1.33	0.20	0.57	0.09	1.12	0.09	–	–
Ti6Al7Nb	1/2/3/4/97V/S	1.04	0.20	0.45	0.05	–	–	1.22	0.11
	1/3/4/97V/S	0.99	0.06	0.40	0.05	–	–	1.01	0.09
	1/3/4/5/97V/S	1.00	0.10	0.44	0.09	–	–	1.11	0.05
	1/2/5/97V/S	1.30	0.20	0.54	0.02	–	–	1.20	0.09

Conclusions

Produced by anodic oxidation layers and their degradation products do not cause cytotoxic effects.
Cells of the SaOS-2 line after incubation with the Ti6Al4C and Ti6Al7Nb alloys extracts with a modified surfaced showed no change in morphology and organization of them. Due to the lack of reference levels of safe, non-cytotoxic effects on cell cultures, concentrations of titanium alloy degradation products obtained in the work concentration can be considered as the reference level.

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STEM CELLS AND THEIR DERIVATIVES – CURRENT PERSPECTIVES IN TISSUE ENGINEERING AND REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 39]

Abstract
Several current approaches in tissue and organ regeneration focus on applications of recent achievements of cell- based therapies and biomaterial sciences. Such combined approaches relying on both components such as stem cells (SCs) with high regenerative potential and new biocompatible scaffolds opens new opportunities in tissue engineering and injured organ treatment.
Several types of SCs with multi- and pluripotent characteristics such as mesenchymal stem cells (MSCs) of various origin and induced pluripotent SCs (iPSCs) have been indicated as potential source of cells for therapy. When combined with optimized biocompatible carriers and scaffolds, such SC fractions become leading targets for cell-based regenerative applications in several tissue injuries.
Although such SC populations have been employed in experimental therapies of several organs injuries as well as in clinical studies, there is still discussion which subpopulation/s would be the most efficient and safe for therapies in humans. The selection of the optimal cell population for tissue regeneration would include predominantly safety aspects as well as major mechanisms of action critical for a specific tissue repair that are provided by specific SC population. Such mechanisms of SC activity includes extracellular vesicles (EVs) release. Such stem cell derivatives may modulate endogenous cell functions in place of transplantation by transferring several bioactive SC- derived molecules including proteins and transcripts.
Thus, the newest trends in tissue regeneration would focus not only on combined applications of biocompatible materials with selected and optimized SC fractions, but also with their bioactive derivatives such as EVs. However, successful applications of SCs and their derivatives in regenerative medicine requires safety, ethical acceptance and therapeutic efficacy, which still need further investigations and optimization.

Acknowledgments
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FABRICATION AND CHARACTERIZATION OF GRAPHENE-LOADED CHITOSAN HYDROGELS WITH CROSS-LINKING GRADIENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 40]

Introduction

Hydrogels can be defined as polymeric networks able to absorb large amount of water. Thanks to the aqueous environment and the rubbery nature, hydrogels resemble native cell environment - the extracellular matrix (ECM). However, mimicking the complex, multi-level structure of ECM is still a challenge in tissue engineering. Various techniques have been applied to create hydrogels with gradients of microstructure, mechanical properties and biosignals: 3D printing [1], cross-linking by photopolymerisation using gradient-mask [2] and microfluidic methods [3]. However, most of these processes require use of advanced equipment.

Chitosan (CS) and other natural polysaccharides are extensively tested in tissue engineering [4,5] due to their similarity to natural tissues. CS-based hydrogels possess superior biological properties but their applicability is limited by poor mechanical properties and stability. In mild acidic conditions, the amino groups of CS get protonated and the polymer can be fully dissolved. When increasing the pH, CS solution forms a hydrogel via secondary interactions.

The aim of this study, was to fabricate CS-based hydrogels with gradient properties by gelation method. To enhance the hydrogel properties, various forms of graphene (GO, rGO, GO-PEG) and tannic acid (TAc, cross-linker) were introduced to polymer matrix.

Materials and Methods

CS (High Mw, DD >90%) and sodium tripolyphosphate (TPP) were obtained from Acros Organics, USA. Lactic acid (LAc, 88%), TAc, NaOH, NaCl were purchased from Avantor Performance Materials Poland S.A. Three types of graphene materials: graphene oxide (GO), reduced graphene oxide (rGO) and PEG grafted GO were prepared in ITME, Poland.

CS solution was prepared by dissolving CS (5% w/v) in 5% LAc. Next, stable suspension of GO, rGO or GO-PEG (0.5% to CS weight) and TAc (10% to CS weight) were added to CS solution. The solutions were homogenized by sonication in water bath, transferred into molds and frozen at -20°C for 24 h. Next, they were immersed in gelling solution at 4°C. Types of gelling solutions are summarized in TABLE 1. Finally, the samples were washed with distilled water and stored for 24 h.

TABLE 1. Gelling system compositions.

Lp.	1st bath	Time	2nd bath	Time
1	5% NaCl + 0,5% TPP	24 h	-	-
2	5% TPP	2 h	10% NaOH	10 min
3	5% TPP	4 h	10% NaOH	10 min

XRD, XPS, ATR-FTIR and SEM were used to characterize graphene materials and the obtained hydrogels. Rheological (rotational rheometer with parallel plates), thermal (DSC) and mechanical (compression test) properties of the samples were examined. Also, *in-vitro* degradation (PBS, 37°C) and bioactivity (SBF, 37°C) tests were carried out.

Results and Discussion

Form and properties of the hydrogels can be easily controlled by the time and composition of the gelling solution. In the first gelling system, the gel was formed within the whole volume of the sample. The second composition resulted in the formation of a dense outer shell and a semifluidic center. After increasing time to 4h, CS solution gelled completely and created multilayered hollow rods. FIG. 1 shows gradient microstructures of CS/GO hydrogel after lyophilisation.

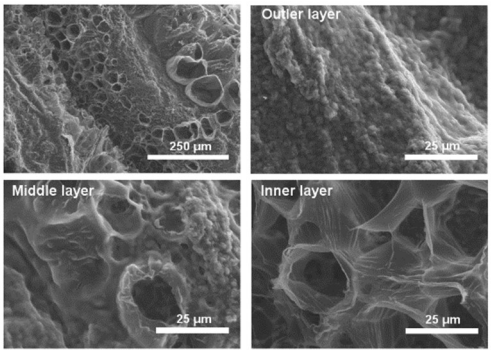

FIG. 1. SEM images of CS/GO hydrogels after gelling in 2nd system (2 h TPP + 10 min NaOH) and freeze-drying.

ATR-FTIR measurements (FIG. 2) confirmed successful cross-linking of CS-based hydrogels by TPP. In addition, nanocomposites modified with different graphene forms exhibited improved stability during PBS incubation test and high *in vitro* bioactivity.

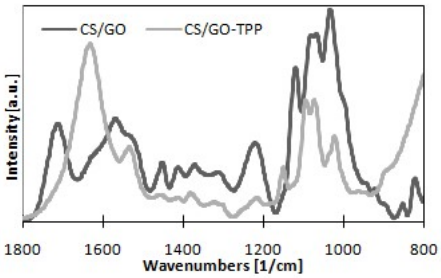

FIG. 2. ATR spectra of CS/GO and CS/GO-TPP.

Conclusions

A novel method was developed to obtain gradient CS hydrogels. Properties of the hydrogels can be controlled by gelling conditions. Gradient nanocomposites loaded with graphene mimic complex structure of ECM and constitute promising materials for tissue engineering.

Acknowledgments

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SELECTION OF ACTIVE FRAGMENTS OF COLLAGEN

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[ENGINEERING OF BIOMATERIALS 148 (2018) 41]

Introduction

Application of natural building blocks for the preparation of three-dimensional matrix useful for regeneration ensure completely biocompatibility of the temporary ECM equivalent. The advantage of these materials used as a scaffolds after deposition of cells, their adhesion, proliferation and differentiation arise from their steady biodegradation leading solely to natural metabolites. The inherent feature of peptides to form stable, specific spatial structures, cause that they can be evaluated as universal platform for the synthesis of nanomaterials with controllable three dimensional framework [1] bearing the properties stimulating the regeneration process [2]. On the other hand, the presence of polysaccharides assure the formation stable three-dimensional matrix. Considering the properties of both classes of compounds, attempts were made to obtain hybrid materials in which fragments of main ECM proteins were covalently attached to alginate and hyaluronate. The application of collagen derivatives was preferred due to the fact that this protein is the fundamental component of the ECM. The preliminary result of study indicate that synthesized hybrid materials can be useful as a scaffolds for regenerative medicine. On the other hand, it is also possible to use both of these components as scaffolds for use in regenerative medicine. At the Institute of Organic Chemistry of the Lodz University of Technology, research to select biologically active collagen I, II and III fragments able to form a new collagen network derivatives useful as a three-dimensional materials was undertaken. The use of biologically active protein fragments instead of whole proteins eliminates the immunogenic properties of the protein and on the other hand allows to modify their stability.

Materials and Methods

At the stage of selection of biologically active fragments of collagen I, II and III, the SPOT technique of synthesis of protein fragments was used. All tested proteins were divided into decapeptide non-overlapping fragments that were used for synthesis of three libraries of immobilized peptides covering whole collagens I, II and III. Chr-1 Whatman filter paper was used as matrix in the study. Cellulose was modified with 2,4-dichloro-6-methoxy-1,3,5-triazine and and Fmoc-Gly according to standard protocol [3]. Syntheses of immobilized peptides were made by automated SPOT [4] methods using as a coupling reagent DMT/NMM/TosO$^-$ [5]. The synthesized peptide libraries were treated with specific antibodies. A standard Dot-blot procedure was used to visualize the immune complexes. Strong immunological complexes were subjected to epitopic mapping with a shift of the reading frame by five amino acid residues towards the N- and C-terminus.

Results and Discussion

Research on the selection of biologically active collagen I, II and III fragments has been carried out. The process is multi-stage and comprised of synthesis libraries covering the entire proteins in the form of decapeptidic fragments immobilized on cellulose. The synthesis was carried out according SPOT technique by using triazine coupling reagents.

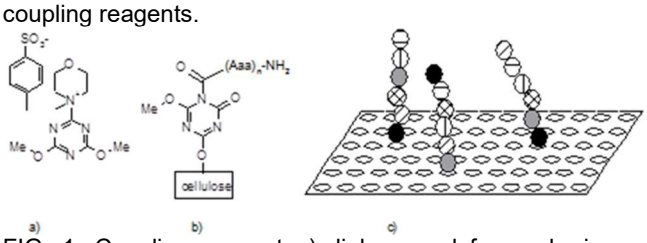

FIG. 1. Coupling reagent a), linker used for anchoring peptides b), and cellulose dot matrix of peptides c) used in the SPOT methodology.

The dot blot assay selected collagen fragments (epitopes) that formed immunological complexes with antibodies. The epitopes feature is the preservation of the proper secondary structure of the native protein.
In the first screening of the 168 element library of collagen I fragments, 33 decapeptides forming strong immunological complexes and 38 fragments with moderate antibody binding capacity were selected (FIG. 2). In the case of collagen II, from the 149 element library of peptide fragments in the dot-blot test, 26 peptides with the ability to interact with antibodies were selected, of which 4 formed strong immunological complexes. From the 147 element library of collagen III fragments, 52 fragments with the ability to interact with antibodies were selected, of which 12 decapeptides formed strong immunological complexes.

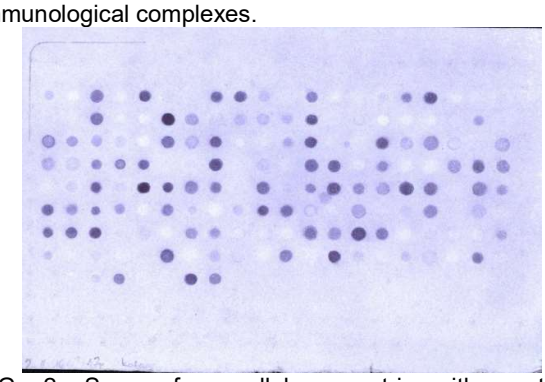

FIG. 2. Scan of a cellulose matrix with peptides embedded on its surface after dot-blot analysis.

After epitope mapping and re-blot testing, a total of 73 fragments derived from collagen I, II, III have been selected, which have the ability to strongly interact with antibodies, and thus these fragments are exposed to the outside of the proteins.

Conclusions

The collagen fragments selected in the screening studies were synthesized on the solid phase and cross-linked to create spatial structures.

Acknowledgments

Financial support: Grant UMO-2015/19/B/ST8/02594.

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INVESTIGATIONS OF HYDROXYAPATITE CRYSTALS IN ORAL CARE PRODUCTS USING THE TEM METHOD

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[ENGINEERING OF BIOMATERIALS 148 (2018) 42]

Introduction

The work presents the possibilities of using transmission electron microscopy to detect the presence of hydroxyapatite, determine the size and shape of its crystals in oral care products. In order to distinguish crystals of hydroxyapatite, the EDS microanalysis was additionally performed.

Materials and Methods

Thirteen oral care products containing hydroxyapatite were tested: 10 tooth cleaners, 1 oral hygiene gel and 1 oral hygiene powder.
The insoluble fraction of product was analyzed. It was obtained by dilution of the product in water and vacuum filtration through filter with a 0.47 μm pore size.
The picture and micrograph for microanalysis were obtained by TEM JEM 1400 (Jeol Co., Japan, 2008).

Results and Discussion

Photographs and micrographs (FIG. 1) of individual elements were superimposed on each other, which allowed for attributing groups of crystals to silica, titanium oxide and hydroxyapatite. In order to improve the clarity, the micrographs were given different colors and colored images were thus obtained.
The detected crystals were subjected to size measurements and their morphology was described.

Conclusions

The applied methodology of photo analysis allowed for the attribution of hydroxyapatite crystals to silica and titanium oxide. The isolation of hydroxyapatite crystals and the determination of their morphology allowed the confirmation of the presence of hydroxyapatite in the tested products. Silica crystals were the smallest in the tested products, and the crystals of hydroxyapatite and titanium oxide were of similar size. The hydroxyapatite crystals had the shape of needles, rolls and plates.

Acknowledgments

The TEM studies were performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, Warsaw, Poland. We used equipment installed within the project sponsored by the EU Structural Funds: Centre of Advanced Technology BIM—Equipment purchased for the Laboratory of Biological and Medical Imaging: a high performance biology transmission electron microscope JEM 1400 (JEOL Co., Japan, 2009) equipped with energy-dispersive full range X-ray microanalysis system (EDS INCA Energy TEM, Oxford Instruments, UK).

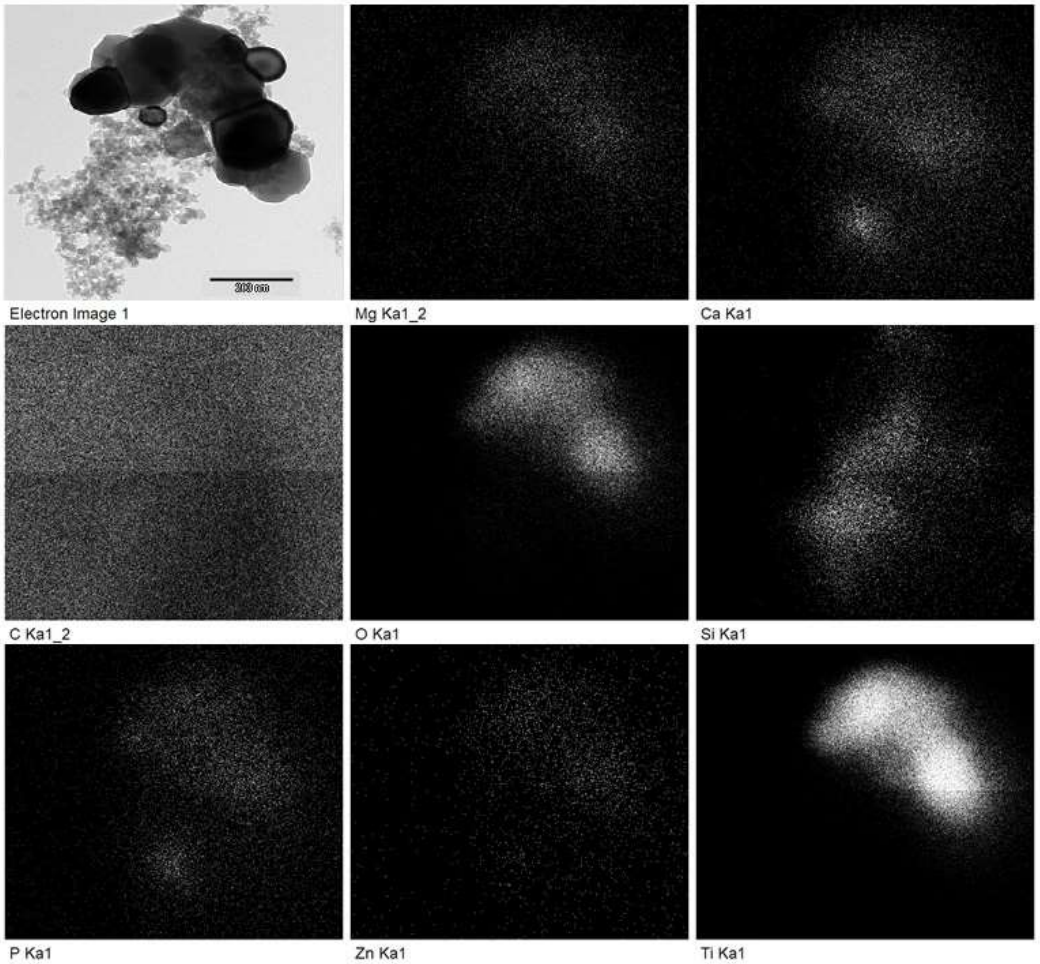

FIG. 1. Picture of TEM and results of microanalysis EDS of GC Mousse paste for magnesium, calcium, carbon, oxygen, silicon, phosphorus, zinc and titanium.

POROUS HYBRID MATERIALS AS POTENTIAL DRUG DELIVERY SYSTEMS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 43]

Introduction

In recent years, numerous studies have been carried out to obtain composites (hybrids) based on hydroxyapatite.
Hybrid materials belong to the new generation of materials, which constitute a homogeneous mixture of inorganic and organic components, not exceeding the 1 μm scale. Thanks to this construction, it is possible to design new materials or modify existing ones that will have features that are not seen separately in individual components [1-5]. In our work, we have developed hybrid materials containing hydroxyapatite (HA) and organic compounds: alginate (Alg), keratin (Ker), chondroitin sulphate (CS) and cellulose (Cel). The Ibuprofen (Ibu), a model drug was additionally incorporated into the material's structure.
The release profile of ibuprofen from the obtained composites was also checked.

Materials and Methods

Four types of granules were analyzed in the work (TABLE 1).The granules were obtained by instilling the water solution of all ingredients in a 1.5% $CaCl_2$ solution. The granules were then dried at 40˚C.

TABLE 1. Granules composition.

	Granules			
	I	II	III	IV
HA	+	+	+	+
ALG	+	+	+	+
Ibu	+	+	+	+
	-	CS	Ker	Cel

SEM images of the granules were taken and also release of the ibuprofen from the granules to the pH 7.4 buffer was examined.
The released ibuprofen was determined by HPLC method and release profile over time was plotted.

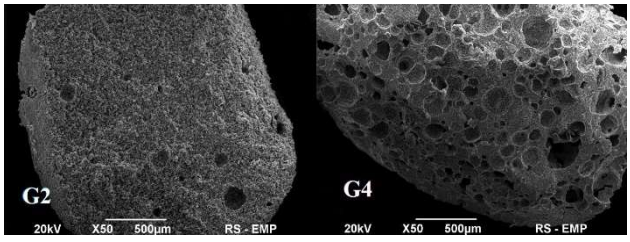

FIG. 1. SEM photos of granules showing cross-section through granules G2 and G4.

Results and Discussion

FIG. 1 shows representative SEM images of cross-sections through granules. SEM images shows that the most compact structure was observed in the granules containing CS. On the other hand, granules that make Cel and Ker possess numerous micropores. It should be noted that the porosity may affect the release of the therapeutic substance from the granules.
The studies of release of Ibu by HPLC method show that during the first 12h granules I, II and IV released the whole of Ibu, while the release process from granules III containing Ker was much slower.

Conclusions

The obtained hybrid materials containing: hydroxyapatite (HA) and organic compounds: alginate (Alg), keratin (Ker), chondroitin sulphate (CS) and cellulose (Cel) may be used as drug delivery systems.
The addition of Ker, Cel and CS affects the internal structure of the obtained granules. Their interior can be compact or contain numerous micropores. The addition of Ker, Cel and CS also affects the release time of Ibu.
Keratin addition significantly prolonged the release time of ibuprofen.

Acknowledgments

This work was supported by the research programme (UMO-2016/22/E/ST5/00564) of the National Science Center, Poland.

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COMPOSITE NiTi/MULTI-PHASE LAYERS FOR MEDICAL APPLICATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 44]

Introduction

Despite the good biotolerance of the NiTi alloy by humane organisms, studies on the surface functionalization of these alloys is being carried out. On the surface of the alloys, layers are formed, which form barrier against nickel migration and also enable faster osseointegration or contain antibacterial substances [1-3].
In presented work the multi-phase layers was created on the top of NiTi alloy for its potential use in medicine and/or veterinary.

Materials and Methods

Surface of a commercial NiTi shape memory alloy was covered with multi-layer in two steps. First, the NiTi alloys was oxidized using glow discharge technique. Next, the top of oxidized layer was covered by mixture, consisted of chitosan and silver, in one electrophoretical process (voltage: 20-60V; time: 60-120s).
Received multi-phase layers were studied by X-ray diffraction (X'Pert Pro diffractometer). Surface was observed by scanning electron microscope (JEOL JSM 6480).

Results and Discussion

X-ray diffraction patterns measured for as-received surface of NiTi revealed presence of the R-phase (FIG. 1). This phase is an intermediate one and appears as a first step in two-steps martensitic transformation, where the B2 parent phase transforms to the martensite B19'. Titanium willingly forms oxides due to its higher affinity for oxygen than nickel. The oxidation was done using glow discharge technique. The alloy surface has been cleaned, polished and prepared to deposition of the oxide layer. After processing, a thin layer of titanium oxide was formed on the surface. In order to confirm structure of the oxide, the X-ray grazing incidence beam diffraction technique was used.

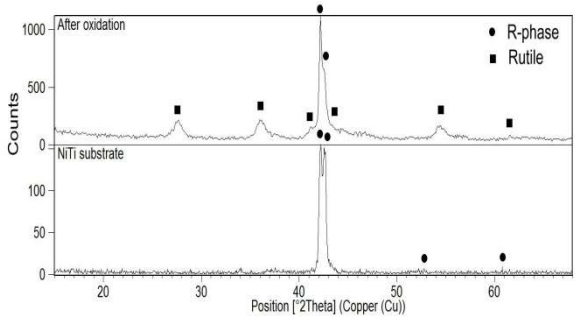

FIG. 1. Comparison of X-ray diffraction patterns measured for as-received NiTi alloy and after its oxidation.

Diffraction pattern measured at the constant incidence beam angle (0.5 deg.) showed presence of diffraction lines, which are representative for titanium oxides – rutile (ICDD card no 77-0441). Apart of that still the presence of the R-phase was stated (FIG. 1).
The oxidized surface of NiTi alloy was a substrate for deposition of a multi-phase layer containing chitosan and silver nanoparticles. FIG. 2 shows example of SEM image observed for NiTi surface after chitosan/silver layer deposition. The silver nanoparticles were well distributed and covered by chitosan.

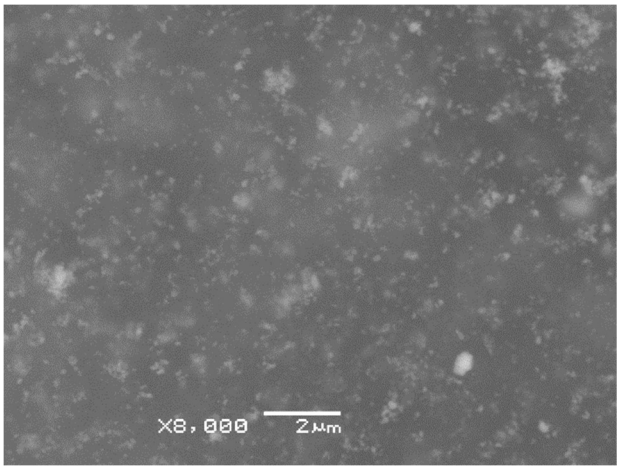

FIG. 2. SEM image observed from the top of modified surface of NiTi alloy after chitosan/Ag deposition.

The measured X-ray diffraction pattern, for a coated alloy with a chitosan/silver layer, contains diffraction lines characteristic for silver. In addition, the increased half-width of diffraction lines, representative for the silver, indicates that its nanometer size is maintained. The presence of chitosan, in the layer, confirms the anomaly of the background between an angle of 20 and 30 degrees. This effect, originating from the X-ray radiation scattering and indicates the amorphous nature of chitosan.

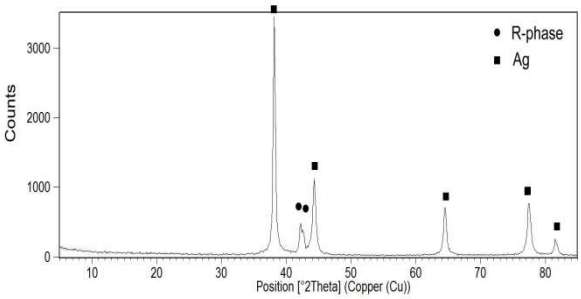

FIG. 3. X-ray diffraction pattern measured for oxidized NiTi alloy covered with Ch/Ag composite.

Conclusion

Combining the glow-oxidation technique with electrophoresis extends the ability to modify the surface of NiTi alloys for applications as a material for implants in medicine and veterinary medicine.

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VARIOUS IONIC SUBSTITUTIONS IN HYDROXYAPATITES - PHYSICOCHEMICAL STUDIES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 45]

Introduction

The mineral part of bone and teeth are mainly composed with biological apatite, nanocrystalline multi-substituted carbonated hydroxyapatite containing various ions (i.e. Na^+, K^+, Mg^{2+}, Mn^{2+}, Zn^{2+}, HPO_4^{2-} or SiO_4^{4-}, etc.) [1]. Hydroxyapatite (HA) with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is commonly used as bone substitute and biomaterial for orthopaedic and dental applications. The crucial property of synthetic HAs is the ability to ionic substitution. Therefore, ionic substitution may be a tool to synthesize materials with high biocompatibility with mineralized tissues [2].

Moreover, it should be noted that these substitutions may provoke changes in physicochemical and biological properties [3,4].

The main aim of this work was to synthesize HA enriched in different ions (i.e. SeO_3^{2-}, Zn^{2+}, BO_3^{3-}, Sr^{2+}, SiO_4^{4-}) and to provide detailed study on their chemical structure and physicochemical properties.

Materials and Methods

Hydroxyapatites containing various ions were synthesized using two different ways: standard, wet method and solid-state method. The obtained powders were examined using various analytical methods: powder X-ray diffractometry (PXRD), infrared spectroscopy (FT-IR), transmission electron microscopy (TEM) and solid-state magnetic resonance spectroscopy (ssNMR). The elemental analysis were performed by using wavelength dispersive X-ray fluorescence spectroscopy (WD-XRF).

Results and Discussion

PXRD diffractograms have shown the significant differences in degree of crystallinity among the substituted HAs. Different sizes and shapes of crystals were confirmed by TEM microscopy. FT-IR and ssNMR spectroscopy allowed to analyse the location of “foreign” ions. Moreover, the hydrated surface layer was detected using ^{31}P MAS NMR experiments.

Conclusions

The powders of substituted hydroxyapatites were successfully synthesized and detailed physicochemical analysis were carried out. The studies have shown that ionic substitution may have an important impact on the crystal sizes and shapes, crystallinity index or solubility and the development of hydrated surface layer. The obtained results shown that spectroscopic methods may be an appropriate way to study apatitic materials.

Acknowledgments

This work was supported by a research grant (Project NCN UMO-2016/22/E/ST5/00564) from the National Science Centre, Poland.

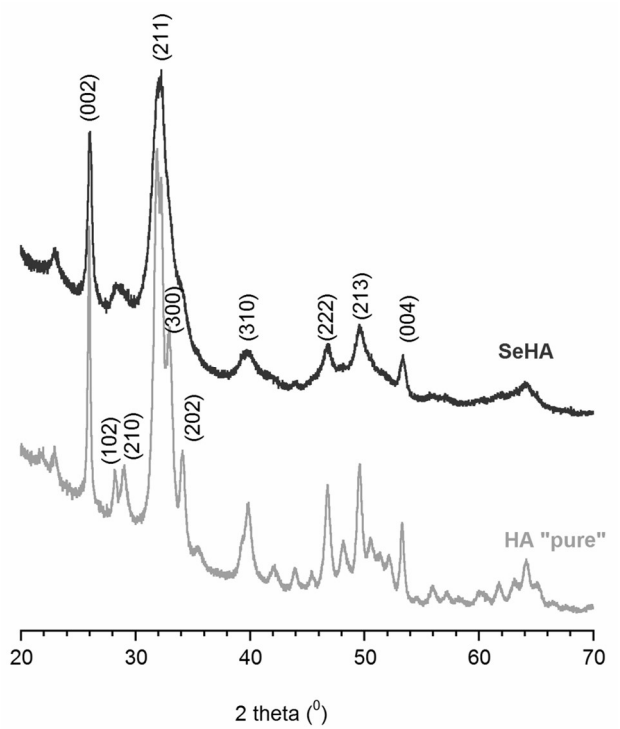

FIG. 1. PXRD of the HA and Se-HA samples.

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SYNTHESIS OF SILVER AND GALLIUM DOPED HYDROXYAPATITES FOR POTENTIAL ANTIBACTERIAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 46]

Introduction

Hydroxyapatites, due to their biological, chemical and physical properties, are used for years in bone and dental surgery. These biomaterials exhibit biocompatibility with bone tissue as well as they do not show negative properties like cytotoxicity and sensitizing activity [1-3].
Implementation of these medical materials into organism during a surgical operation carries a potential risk of bacterial infection inside operated location, which in turn may lead to systemic infection. This kind of danger imposes using appropriate antibiotic therapy, where medications are administered intravenously or orally. Unfortunately, above-mentioned routes of administration enforce using high dosages of antibiotics due to weak vascularity of bone tissues, which can cause serious adverse events and can destroy natural microbiota of human body.
Thanks to hydroxyapatite's ability to easy adsorption many of drug substances, it could be used as carrier for antibiotics or ions with antibacterial properties [4,5]. Following work was focused on synthesis of hydroxyapatites doped with two such ions - silver and gallium, and afterwards on studying properties of obtained biomaterials.

Materials and Methods

Hydroxyapatites were synthesized by wet precipitation method and solid-state method. The following reagents were used: $Ca(NO_3)_2{\cdot}4H_2O$, $(NH_4)_2HPO_4$, $AgNO_3$, $Ga(NO_3)_3{\cdot}3H_2O$, $NH_3{\cdot}H_2O$ in wet precipitation method and $CaCO_3$, $(NH_4)_2HPO_4$, Ag_2CO_3, Ga_2O_3 in solid-state method.
In wet precipitation method adequate amount of $Ca(NO_3)_2{\cdot}4H_2O$ and $AgNO_3$ or $Ga(NO_3)_3{\cdot}3\ H_2O$ (or both) was dissolved in 500 ml of distilled water and $(NH_4)_2HPO_4$ in 50 ml of distilled water. Next, diammonium hydrogen phosphate solution was added drop-wise to calcium nitrate solution. Afterwards ammonia solution was added to adjust pH to 10. Reaction mixture was heated to 70°C and stirred for 2 h and after that time it was left to age for 24 h. Finally, obtained product was filtered and washed with distilled water to eliminate ammonium nitrate and dried at 100°C for 24 h.
In solid-state method adequate amount of $CaCO_3$, $(NH_4)_2HPO_4$ and Ag_2CO_3 or Ga_2O_3 (or both) was added to milling container and then milled for 30 min. Product was formed into tablet and sintered in 1100°C.
In total, 10 hydroxyapatites doped with silver, gallium or both ions were obtained.
Following physicochemical methods were used to investigate the obtained materials: Powder X-ray Diffraction (PXRD), Solid-state Nuclear Magnetic Resonance (ssNMR), Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscope (TEM). In addition, biological study to determine cytotoxicity of synthesized materials was performed. The neutral red uptake (NRU) cytotoxicity test was performed on the basis of ISO 10993 guideline Annex A [6] with BALB/c 3T3 clone A31 mammalian cell line.

Results and Discussion

PXRD, ssNMR and FTIR experiments allowed to determine that synthesized materials are hydroxyapatites. Using the wet method, the powders were obtained as nanocrystals, what is visible on TEM photos (example on FIG. 1). The samples from solid-state method are microcrystalline. PXRD diffractograms confirm high crystallinity of the samples obtained by dry method.
Cytotoxicity tests exhibit that almost all the synthesized biomaterials were non-cytotoxic. Only in case of the sample with highest concentration of silver ions cytotoxicity occurred.

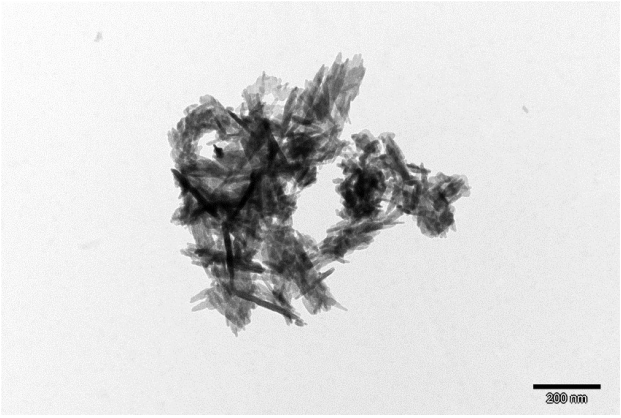

FIG. 1. TEM image of nanocrystals of the synthesized hydroxyapatite doped with silver ions.

Conclusions

In following work, 10 hydroxyapatites doped with silver or gallium ions (or both) were synthesized. Their identity and chemical structure were confirmed thanks to various physicochemical studies. Majority of synthesized biomaterials didn't show cytotoxic properties. Currently antibacterial properties of these materials are under investigation. Results of these studies will allow to determine possibility of apply obtained materials in the cases mentioned in the Introduction.

Acknowledgments

This work was supported by the research programme (UMO-2016/22/E/ST5/00564) of the National Science Center, Poland.

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ASSESSMENT OF SELECTED STRUCTURAL PROPERTIES OF ORTHODONTIC ARCHES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 47]

Introduction

The most important requirements set for the arches are first of all its high biocompatibility, biostability and non-toxicity. To this day, the aspect that raises the most doubts in using the nickel-titanium arches in medicine is the risk of the nickel ions being released into the patient's body. Nickel can lead to allergic reactions in patients. It is the cytotoxic, genotoxic and carcinogenic element. Due to occurrence of the material heterogeneity and the high factor of the orthodontic arch roughness, the rate and quantity of the released nickel can gradually increase. Therefore, the applied material is still a subject of many experiments, and in the orthodontics it is applied only in a short-term therapy [1-3]. Due to this, in the present work, a particular attention was paid to the possible impact of orthodontic wires operation on quality of the material and function of human body.

Materials and Methods

For the planned studies the superelastic orthodontic arches from *Adenta* and *Ormco* producer were used. Each of them was made of the nickel-titanium alloy (NiTi) and it belonged to the IInd generation. The cross section of a singular sample in the initial state was rectangular of the 0,016" x 0,022" size.
The randomly selected orthodontic wires were divided to smaller sections and included in the resin. In order to reveal microstructure of the studied samples of orthodontic arches, the HF - HNO_3 – CH_3COOH agent was applied.
The tests were divided into two main stages. The first one concerned the tests on the orthodontic wire samples in the initial state. The purpose of the second one was testing the arches properties after 4-weeks exposal in the patient's mouth. The performed tests involved analysis of the chemical composition, microstructure tests before and after the surface etching, as well as analysis of the material contamination degree according to the Polish standard PN-64 / H-0410.

Results and Discussion

For the samples in their initial state from Adenta and Ormco, the analysis of the chemical composition, have shown about 55 % of nickel and 45 % of titanium.
Microstructure of the orthodontic arches material in the as delivered state from Adenta is characterised with appearance of clear needles of martensite. The similar situation is observed in the case of materials provided by Ormco producer.
The characteristic phase of the fine acicular martensite has also been observed in the case of samples after the 4-week exposal in mouth.

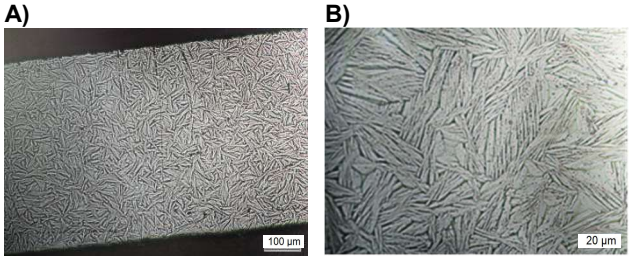

FIG. 1. The microstructure of the random selected nickel-titanium orthodontic arch from Adenta, where A) as delivered state, B) after the 4-week exposal in mouth. (LM)

The non-metallic inclusions, of the oxides and silicates type, have been observed in case of assessing the purity of orthodontic arches received from Adenta and Ormco in their initial state (TABLE 1). The microscopic tests of, randomly selected wires have shown appearance of the first of all the silicates of globular shape (KN), several locally laid non-brittle silicates (KK), and few point oxides (TP). The number and type of the inclusions was random and different for the randomly chosen arch.

TABLE 1. Results of the assessment of the contamination degree of the orthodontic arches from Adenta (A1,A2,A3) and Ormco (O7,O8,O9) producer - the non-etched, as delivered state, where TP- the point oxides, KN – the point silicates.

Brand Name	Sample	Indicator of non-metallic inclusion	
		TP	KN
Adenta	A1	2,00	3,90
	A2	3,70	2,70
	A3	3,40	2,80
Ormco	O7	1,50	1,75
	O8	2,80	1,10
	O9	1,30	3,00

A curious situation occurs when evaluating cleanliness of the material after its 4-week exposal in a patient's mouth. Independent of the arch producer, the tabular result summary did not make sense finally. It turned out that each operated arch was characterised with very small number of any non-metallic inclusions.

Conclusions

The observation of microstructure in each of the studied cases has shown presence of the very fine martensite needles. It is confirmed by the super elastic character of the material, among others the Ni-Ti alloys, which is featured by presence of the low-temperature (from about 4 to some 25°C) martensite phase.
The evaluation of the contamination degree for the orthodontic arches material in their non-etched state has shown the clear occurrence of non-metallic inclusions, mainly for the as delivered state. In the metallographic analysis of samples after oral exposure, these inclusions were definitely less. It may be the result of the everyday operation of the whole orthodontic appliance. Thus, some part of the material gets into the patient's organism, including the non-metallic inclusions, as well as the already mentioned allergenic nickel.

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PREPARATION OF PSYLLIUM HUSK POWDER BASED MICROPOROUS COMPOSITE SCAFFOLDS FOR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 48]

Introduction

Tissue engineering is a mixed discipline that majorly focuses on the recreation and regeneration of diseased or damaged tissues [1], [2]. Tissue engineering scaffolds are an absolute necessity that provides a conducive environment for cell growth and reproduction with respect to its three-dimensional structure, pore size, strength, cell attachment, degradation rate etc [3], [4]. Plantago ovata or psyllium husk is one of the most widely used and commercially available plant-derived polysaccharides in Indian markets [5]. It has been used in many biomedical applications because of its ease of availability, low cost, non-toxicity, biodegradability and safety [6]. Gelatin, a versatile and naturally occurring biopolymer helps in the modulation of cell adhesion because of the presence of cell adhering moieties [7], [8]. 1-ethyl-3-(3-dimethylaminopropyl)-1-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) has been used as a cross-linking agent as it introduces an amide or an ester bond between the functional moieties of the biopolymers. The main aim of this study is to demonstrate the fabrication and characterization of psyllium husk powder and gelatin based three-dimensional microporous scaffolds using two different methods of drying thus supporting its potential for tissue engineering applications.

Materials and Methods

Psyllium husk powder and gelatin composite scaffolds were prepared by mixing them in the ratios 50:50, 75:25 and 100:0 (w/w) respectively, in water. The mixtures obtained were cross-linked using EDC-NHS coupling reaction followed by a drying step. In the present study one set of scaffolds were dried for two days using a desiccator connected to a vacuum pump accompanied with a liquid nitrogen dip for complete drying. The other set of scaffolds was cross-linked using the same procedure as above except it was dried for two days in a freeze-drier.

Scanning electron microscopy analysis was carried out to determine the porous architecture of the scaffolds after respective drying steps. Cell culture studies were performed with L929-RFP (red fluorescent protein) mouse fibroblast cells to identify cell viability, growth and cell-cell communication within the fabricated scaffold.

Results and Discussion

From the results in FIG. 1 it is observed from the digital images of the fabricated scaffolds that the set that was dried in a vacuum desiccator and exposed to liquid nitrogen exhibits a contracted or deflated physical appearance (FIG. 1a) whereas the set of scaffolds subjected to freeze-drying protocol maintain the physical aspect and integrity of the scaffold (FIG. 1b). The difference in the physical attributes of the scaffolds prepared from two distinct procedures is due to the fact that vacuum desiccation followed by a dip in liquid nitrogen removed all the moisture from the scaffolds thereby leaving no void space within the scaffolds for it to be considered as microporous as highlighted from the SEM images (fig 1a). On the contrary, the freeze-drying procedure exploits water content as a template to create microporous structures within the scaffolds thus maintaining its porous morphology.

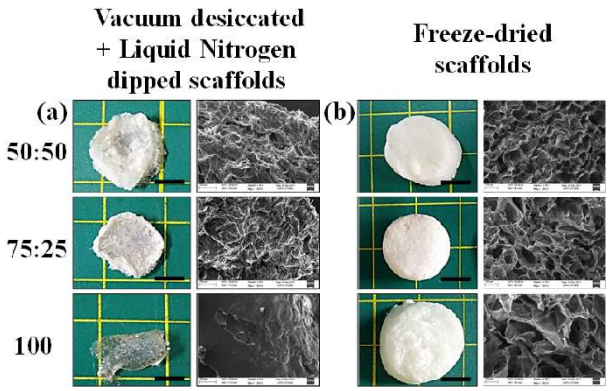

FIG. 1. The above picture represents the digital images of fabricated scaffolds by utilizing the two methods for drying the scaffolds and the SEM micrographs that depicts the porous architecture of the scaffolds. Scale bar: 0.5 cm

Conclusions

The outcomes of the conducted study led us to the conclusion that EDC-NHS crosslinked scaffolds fabricated by freeze-drying step exhibit superior porous structure in comparison to those dried in a vacuum desiccator accompanied with liquid nitrogen dip. Structure and integrity of the scaffolds were better maintained in those developed using a freeze-drier unlike the other set that presented a shrunken appearance. Therefore, in the light of the above facts, it was inferred that scaffolds fabricated by the freeze-drying process were more suitable for cell-culture and tissue engineering applications.

Acknowledgments

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THE INFLUENCE OF COLLAGEN FROM VARIOUS SOURCES ON SKIN PARAMETERS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 49]

Introduction

Collagen, as the main component in extracellular matrix and connective tissue, is the most abundant protein in mammals [1]. Currently, at least 29 types of collagen have been discovered [2,3]. The various types of collagen differ in amino acid sequence, structure and function. Collagen proteins are characterized by a triple-helix structure in which three polypeptide chains are supercoiled into a triple helix. These chains are formed of a repeating triplet: Gly-X-Y, where Gly is glycine, X is generally proline and Y is hydroxyproline [3].

This biopolymer provides the structural integrity and elasticity of the connective tissue and its tensile strength. The skin contain mainly type I, III and V collagen, which determine the tension, elasticity, durability and hydration of skin [4].

Type I collagen is distributed in bone, skin, tendon, ligaments, cornea and other organs [1]. For that reason, it is usually extracted from many natural sources like rat tail tendons or fish wastes.

The aim of this work was to compare the influence of collagen extracted from different species (rat tail tendons, fish scales of northern pike (*Esox lucius)* and skin of *Brama australis*) on skin parameters such hydration, colour, pH and skin's barrier quality.

Materials and Methods

Collagen from rat tail tendons [5] as well as collagens from fish tissues – scales of *Esox lucius* [6] and skin of *Brama australis* [7] were prepared in our laboratory.

Afterwards, 0,1% solutions of each collagen were prepared and applied on the forearm skin. Then, the evaluation of skin condition after application of collagen solutions was made, including hydration, pH, colour and skin's barrier quality.

The hydration level of the skin surface (*stratum corneum*) was determined using Corneometer CM 825 (Courage+Khazaka, Germany). Skin's pH was tested using pH-meter (Elmetron, Poland), skin's barrier quality (TEWL-Transepidermal Water Loss) was examined using Tewameter TM 300 (Courage+Khazaka, Germany) and skin colour was measured by Skin-Colorimeter CL 400 (Courage+Khazaka, Germany).

The measurements had been taken on the skin surface in three places before application and after 10, 20, 30, 60, 120 and 180 minutes from application of the collagens solutions. The results of this measurements were averaged and standard deviation was calculated. All measurements were performed in the laboratory in controlled temperature and humidity conditions (20-22°C, relative humidity 40-60%).

Results and Discussion

The application of obtained collagen from rat tail tendons and fish tissues (marine *Brama australis* and fresh water *Esox lucius*) had initially deteriorated the skin's barrier quality manifesting itself as increase in TEWL. The highest TEWL value was observed after application of collagen from rat tail tendons. The level of TEWL had returned to the initial level 120 minutes after application of collagen solutions from rat tail tendons and *Esox lucius* scales and after 180 minutes in the case of collagen from the skin of *Brama australis*. The solution of collagen extracted from the scales of *Esox lucius* improved the skin's barrier quality – 180 minutes after application of this solution the level of TEWL decreased below the preliminary level.

A slight redness of skin appeared after the application of collagen solutions. Collagen from rat tail tendons made the skin the most red and irritated. Solely after 120 minutes from the application of collagen solution from *Esox lucius* scales the skin colour had returned to the initial level.

The application of collagens solution had improved the hydration of the outer skin layers. After 30 minutes the level of hydration of the skin surface decreased, however, within three hours of the study it remained at a higher level than the initial one regardless of the source of collagen. The solution of collagen from *Esox lucius* scales have the best long-term moisturizing properties. The increase in TEWL could have an indirect impact on corneometric measurements.

Application of rat collagen solution had increased the skin pH, while solution of fish scale collagen had slightly decreased the pH of skin.

The most harmful effect on skin parameters was observed after application of rat tails collagen solution. Collagen extracted from scales of *Esox lucius* showed the most favourable effect on the skin parameters.

Conclusions

The source of collagen have the significant influence on its effectiveness. The greatest virtues for human body were observed in the case of fish collagen extracted from *Esox lucius* scales.

Collagen, due to its biocompatibility, biodegradability and non-toxicity, is widely used for cosmetic, pharmaceutical and biomedical applications. Fish collagen may be a good base for the production of collagen matrices for the skin applications (e.g. for wound dressings), because it exhibit a positive active effect on the skin. Moreover, other active substances can be incorporated into this matrices. For that reasons, fish collagen may be an attractive alternative to mammalian collagen for biomaterials.

Acknowledgments

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MODIFICATION OF GRAPHENE OXIDE AND REDUCED GRAPHENE OXIDE WITH INORGANIC NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 50]

Introduction

Nowadays a lot of scientific effort is focused on the development of new materials for the biomedical use (e.g. for tissue engineering [1] and cancer treatment). One of the most promising material in this field are graphene derivatives: graphene oxide (GO) and reduced graphene oxide (rGO). GO is a defected graphene (carbon layer with one atom thickness arranged in hexagonal crystal lattice), where defects are formed because of reactive oxygen functional groups bonded to the surface. Great interest of GO is due to its physicochemical properties which enable its modification with different biomolecules. This, in turn, extend possibilities for interaction with different types of cells and tissues. The crucial fact is that GO was found to be nontoxic and biocompatible towards different cell lines, even human mesenchymal stem cells [2].
The aim of this study was to obtain composites of GO and rGO with inorganic nanoparticles (NPs): Ag, Ag_2O, SiO_2, hydroxyapatite and TiO_2. These inorganic particles were used due to their biomedical activities.

Materials and Methods

Graphene oxide was prepared via modified Hummers method and reduced graphene oxide was obtained through GO reduction with ascorbic acid and at a temperature of 90°C.
Ag nanoparticles were synthetized in three routes: by $AgNO_3$ reduction with ascorbic acid, with sodium borohydride and polyphenon. Ag_2O was prepared during the reaction of $AgNO_3$ with NaOH solution. To prepare TiO_2, titanium isopropoxide was used as a precursor. Hydrolysis of TEOS led to SiO_2 formation. As a precursors for hydroxyapatite synthesis, $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ were used. Excluding the last one, each kind of NPs was obtained directly in GO or rGO water suspension to provide better distribution on their flakes. The molar ratio between GO (and rGO) and NPs was 1: 0.08.
Scanning Electron Microscopy (SEM) was used to evaluate morphological properties of the synthetized materials. Raman spectroscopy and X-Ray Diffraction (XRD) were used to confirm the chemical structure of prepared materials.

Results and Discussion

Surface topography and distribution of NPs on GO and rGO surface was observed with the use of SEM (FIG. 1).
Pure NPs as well as NPs deposited on GO and rGO flakes were studied. Each kind of the synthesized NPs was of diameter below 100 nm what was stated during SEM measurements. The used 1: 0.08 molar proportion of GO and rGO to NPs occurred to be proper to provide good distribution of NPs on flakes and not to block the whole GO and rGO surface.

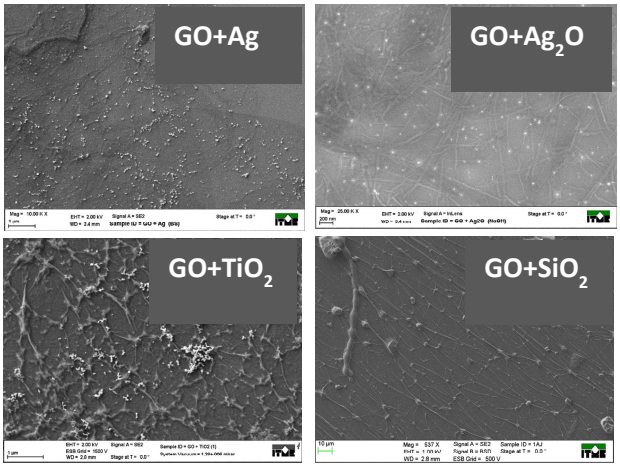

FIG. 1. SEM images of GO with NPs.

XRD measurements were conducted in order to show the chemical and crystal structure of inorganic nanoparticles. It confirmed that proposed synthesis methods led to obtain intended materials (FIG. 2).

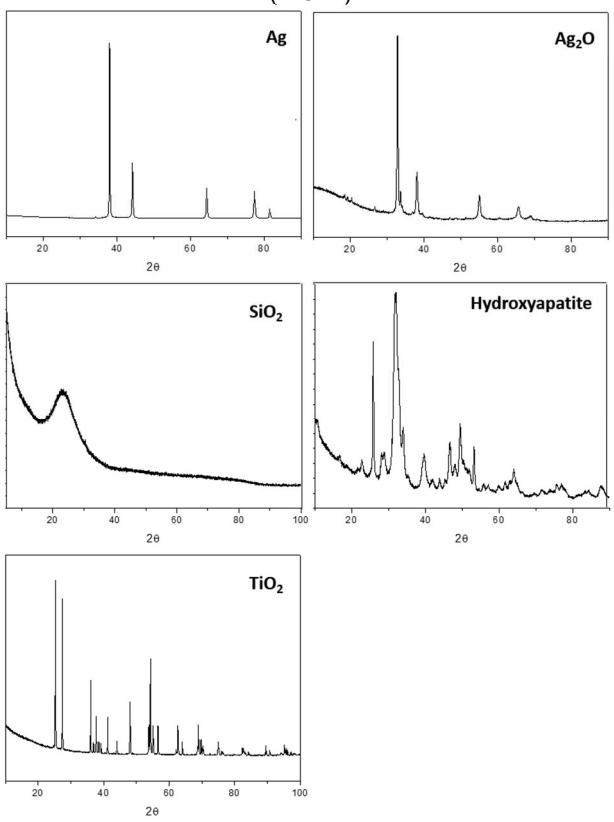

FIG. 2. XRD spectra of the synthesized NPs.

Conclusions

Different composites of GO and rGO with inorganic nanoparticles were synthesized. This work has shown that it is possible to obtain well distributed NPs on the surface of graphene derivatives flakes. Selected NPs are of great importance for bone tissue regeneration. Therefore the composites can be tested e.g. as a platform for tissue culturing and engineering.

Acknowledgments

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APPLICATIONS OF FLAKE GRAPHENE IN TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 51]

Introduction

Graphene, as the material built primarily, or only (depending on the method of production) of the carbon atoms, which naturally occur extensively in all living organisms, seems to open up new possibilities for the fields of science associated with biology and medicine. Graphene, like all other allotropes of carbon, is considered to be biocompatible. The graphene family includes members such as graphene oxide (GO), reduced graphene oxide (rGO), graphene nanoplatelets (GNP) and functionalized graphenes containing various organic molecules or inorganic nanoparticles. Currently the investigations of biomedical applications of graphene are conducted in several main directions: drug and gene delivery, anticancer therapy, tissue engineering, biosensing and bioimaging [1]. To enhance stem cells growth on graphene scaffolds the graphene must undergo biofunctionalization. It was proved that decoration graphene flakes by gold nanoparticles induces osteoblast differentiation. Moreover the shape and size of metal nanoparticle impact on the differentiation process [2].

Materials and Methods

Graphene oxide was prepared by Marcano method. To obtain reduced graphene oxide several “green reductors” were used: citrate acid, glucose and amine compound. Both GO and rGO materials were modified by gold nanoparticles (AuNPs). To obtained GO/AuNPs composites metal nanoparticles were participated as a result of reduction process. After purification the colloidal suspension was combined with pure graphene oxide solutions and mixed using magnetic stirrer and mild sonication. To prepare rGO/AuNPs composites gold ions as hydrogen tetrachloroaurate(III) hydrate, ($HAuCl_4 \cdot 4H_2O$, 99.9%) were introduced to GO solutions. After adding the bioreducer, a simultaneous reduction of GO and gold ions occurred. Next, the material was purified by repeated centrifugation and final dialysis.
Elemental analysis was performed to estimate the level of reduction and oxygen content in rGO. Fourier-transform infrared spectroscopy (FTIR) allowed to determine functional groups in rGO materials prepared by reduction by various “green compounds”. X-Ray Diffraction (XRD) spectroscopy was used to examine the gold nanoparticle sizes and structure. Scanning Electron Microscopy (SEM) was used to investigate the uniformity of metal nanoparticles distribution on the graphene flakes.

Results and Discussion

The starting material for reduction and further modifications were GO flakes with diameter about 10µm (FIG. 1). The chemical composition of rGO depends on nature of reducer. By varying the synthesis conditions and the used reducers, metal nanoparticles of various shapes and sizes can be obtained. Gold nanoparticles were produced in the form of spheres with diameters of 20 nm (FIG. 2), 40 nm (FIG. 3) and 70 nm as well as rods with a length of 70 nm.

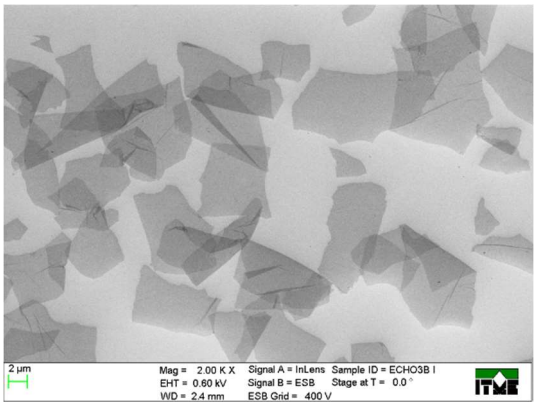

FIG. 1. SEM image of GO.

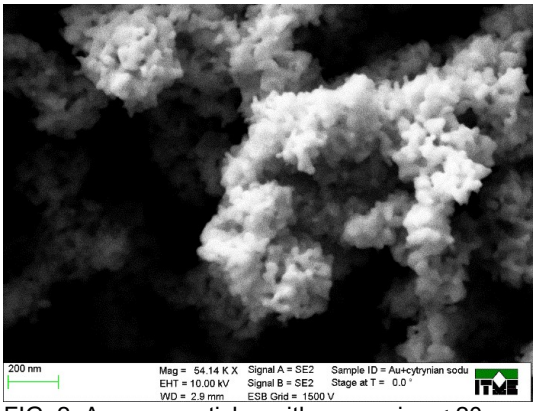

FIG. 2. Au nanoparticles with mean size < 20 nm.

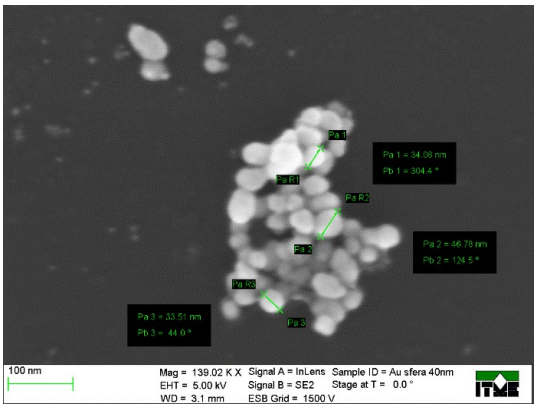

FIG. 3. Au nanoparticles with mean size 40 nm.

Conclusions

In this work we successfully developed green reduction of GO and gold ions to obtain rGO, AuNPs, GO/AuNPs and rGO/AuNPs composites intended for cartilage tissue cell growth. The citric acid, glucose and amine were used as biocompatible reducers. We expect that further biological studies will confirm that the graphene materials and composites produced with the use of amine compound will have great impact on cartilage tissue regeneration.

Acknowledgments

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

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[ENGINEERING OF BIOMATERIALS 148 (2018) 52]

Introduction

Nowadays, one of the most common problems connected with the human healthcare are cartilage tissue failures. This is related to the fact that people live longer and are more physically active. The articular cartilage is exposed mainly to strong shear and compression stresses even during basic activities such as walking. In addition it has very low self-regeneration capacity. In order to deal with this issue, new solutions of tissue engineering are proposed [1].

3D scaffold needs to meet some requirements, such as proper mechanical properties, biocompatible chemical composition, biodegradability and porosity. Good regeneration process of cartilage depends on these issues [2].

The most commonly used materials for this purpose are hydrogels. The main advantages of these materials are the structure similar to living tissue, biocompatibility, and ability to absorb a large amount of water. They can support viability of chondrocytes and synthesis of ECM [1].

Recently, alginate hydrogels became very popular for various biomedical applications. Sodium alginate (SA) is a natural polysaccharide obtained from brown algae. It consists of β-D-mannuronic acid (block M) and α-L-guluronic acid (block G) connected by glycoside bond (FIG. 1) [3].

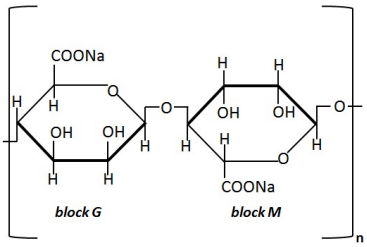

FIG. 2. The structure of sodium alginate.

In the presence of divalent cations (mainly Ca^{2+}) alginate transforms into a gel with the so-called "egg-box" structure [3].

Despite of many advantages, sodium alginate shows poor mechanical properties, strong hydrophilicity, poor thermal stability and limited bioactivity; therefore, its modification is needed [2].

In this work, sodium alginate was reinforced by graphene oxide (GO) and hydroxyapatite (HAp).

Interaction between sodium alginate and GO is possible between functional groups (such as carboxyl, hydroxyl and carbonyl) on the surface of the GO and alginate chains. Ege et al. found that that addition of GO improves the mechanical, biological and rheological properties of the polymer [4].

In case of HAp, it is desired to provide a good connection with the subchondral bone, especially with defects in the bulk of cartilage. It is the result of high biocompatibility, bioactivity and good osteoconductive properties as well as immunogenic one. Moreover, the growth of HAp surface results in advantageous changes on the biomaterial surface, what improves biological properties such as adhesion, proliferation and osteointegration [5].

Materials and Methods

Sodium alginate in the form of powder *(Acros ORGANICS)*, dehydrated calcium chloride in the form of powder *(POCH Avator Performance Materials Poland S.A.)*, graphene oxide in the form of paste *(Institute of Electronic Materials Technology)* and hydroxyapatite *(mkNano)* have been used in hydrogels preparation.

Two series of samples (one with GO and one with HAp) were prepared in the following way: (1) aqueous solution of sodium alginate (25 ml, conc. 3%) was poured into a polypropylene container; (2) GO of concentration 0,1-3% and 1-30% of HAp was dispersed into 5 ml of distilled water and added to the polymer solution; (3) aqueous solution of calcium chloride (90 ml, conc. 0,075M) was added to the polymer solution or polymer solution with additives and mixed.

The sample in the shape of ball was obtained and left for a week in order to obtain fully crosslinked hydrogel.

Properties of manufactured hydrogels such as compression strength, modulus and Poisson ratio *(using ZWICK1435 testing machine)*, chemical stability in *in vitro* conditions *(in PBS and Ringer solution)*, tribology *(using MCR302 rheometer from Anton Paar)* and bioactivity *(in SBF solution by Kokubo method)* were examined. Based on the results, the possibility of usage modified alginate hydrogels in cartilage tissue regeneration was assessed.

Results and Discussion

The obtained results show that the alginate hydrogels modified with graphene oxide nanoparticles as well as hydroxyapatite nanoparticles can be examined by various methods to confirm their regenerative potential. Mechanical and physicochemical properties were investigated to find the appropriate amount of nanoparticles in hydrogel matrix. Modification of alginate hydrogels with HAp and GO significantly affects on mechanical and tribology properties improving the possibility of usage modified hydrogels as materials for frictional surfaces. *In vitro* chemical stability tests confirmed high stability of biomaterials in PBS and Ringer solutions. Bioactivity was examined by SEM observation and EDS analysis which revealed a high content of chlorides on biomaterial surface due to curing method.

Conclusions

The observation of modified hydrogels indicated that sodium alginate hydrogels modified with GO and HAp show regenerative potential for tissue engineering.

Acknowledgments

This research was funded by the National Centre of Research and Development (grant no. STRATEGMED3/303570/7/NCBR/2017).

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IN VIVO EXPERIMENTAL PIG MODEL WITH INDUCED CARTILAGE INJURIES TO ELABORATE TREATMENT OF CARTILAGE AND BONE DEFECTS WITH COMBINED USE OF NEW GENERATION BIOMATERIALS AND STEM CELL FRACTIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 53]

Introduction

Civilization diseases such as diabetes and obesity are serious problems because of numerous and severe complications significantly impacting on the quality of life of patients [1]. One of the serious complications in the course of diabetes and obesity is osteoarthritis leading to degeneration of cartilage tissue [2]. Articular cartilage represents connective tissue with limited regenerative capacity, low level of metabolic activity and poor vascularization. Although methods of treating cartilage defects have been tried to be optimally developed for many years, new and effective methods of treatment are still required [3]. Such novel therapies may include combined use of stem cell fractions settled on new types of biomaterial scaffolds.

Thus, the aim of the research is to develop and use biocompatible materials based on innovative polymers and graphene modifications as scaffolds for human mesenchymal stem cells (MSCs) and ultimately their use in the regeneration of cartilage and bone tissue in patients with osteoarthritis (osteoarthrosis). Thus, an original research model was created on large animals - pigs, which represents one of the most suitable preclinical models translating new treatment procedures to clinic. Pigs as a research model are readily used in medical preclinical studies due to the similarities in both anatomical structure and physiology with human organism. In this project, we used transgenic pigs with a modified immune system allowing for xenotransplantations such as application of human cells into pig tissues with limited risk of immune rejection. This system allows simultaneous examination of new generation biomaterials based on polymer and graphene modifications as scaffolds for human mesenchymal stem cells (MSCs) of various origin. An original surgical model of the production of cartilage defects in pigs for experimental treatment of created injuries was developed.

Materials and Methods

In this project, nine (N=9) transgenic pigs with knockout of α 1,3-galactosyltransferase gene, were used as a research model. The animals were subjected to general anesthesia, supplemented with local anesthesia. The animals were placed laterally on the left side, providing free access to the right knee joint. At the height of the patella on the anterolateral side, skin was cut at a length of about 12 cm. Removal of connective tissue provided access to the knee joint. After cutting the knee capsule, the patella, lateral base of the femur and the base of the tibia bone were visible. Two cartilage injuries, each 6 mm in diameter and 3 - 4 mm deep, were made on the side and medial femoral condyles of the femur. After making the defects, the knee joint was closed with absorbable suture (PLGA 2/0). Subcutaneous tissue was sutured with two layers of absorbable sutures (PLGA 2/0). The skin was sutured with the intradermal hiatus suture, the PLGA 1 sutured suture. After the treatment the animals were provided with painkillers and antibiotic cover for 10 days.

Results and Discussion

The *in vivo* large animal model was developed using transgenic pigs as animals on which the process of healing cartilage defects using human tissues and stem cells can be studied. Cartilage injuries were made in knees of 9 transgenic pigs according to the procedure presented. Obtained easy and quick access to the knee joint, with the possibility of defect in cartilage. The difficulty of the procedure results from the anatomical structure of the knee joint. In the case of the assessment of defect and healing of the cartilage tissue, it is necessary to perform a cavity in the place of the highest tissue load, i.e. on the lateral and medial condyles of the distal femur. This is where the biggest burdens occur. Using the described procedure, access to femoral condyles was obtained with the possibility to perform injuries of any shape and size. Taking into account the size of the femur, it was estimated that a loss of 6 mm in diameter and 3-4 mm in depth would be optimal throughout the thickness of cartilage. During the procedure no difficulties were found in the performance of the cavity. Only one complication was observed in the form of moderate bleeding to the knee joint from the injury site. The bleeds were left for about 3-4 minutes for self-cure. The resulting clots were removed entirely from the knee joint after the bleeding had ceased. The postoperative wound was sutured with four layers of absorbable sutures.

Conclusions

The proposed method of surgical procedure allows quick, efficient access to the knee joint and the performance of surgical cartilage injuries. The use of transgenic pigs with modified immunological system will allow the study of new methods of treatment of cartilage defects in humans. The interspecific system of preclinical trials with use of human stem cells in animal without the risk of rejection seems to be the best for that kind of study.

Acknowledgments

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HUMANIZED TRANSGENIC PIG - AS A PRECLINICAL MODEL FOR *IN VIVO* STUDIES OF CARTILAGE INJURIES AND TREATMENTS WITH NEW GENERATION OF BIOMATERIALS AND STEM CELL POPULATIONS - PRELIMINARY RESULTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 54]

Introduction
Civilization diseases such as obesity and type 2 diabetes and accompanying them complications, including musculoskeletal disorders, such as osteoarthritis (*osteoarthrosis*), are a challenge for modern pharmacology and regenerative medicine [1]. Modern innovative therapeutic treatments includes the use of stem cells and biomaterials. The biomaterials are based on innovative polymers and graphene modifications as scaffolds for human mesenchymal stem cells (MSCs) for the regeneration of cartilage or bone injuries. To introduce mentioned treatments in regenerative medicine practice, an adequate research models are required, e.g. transgenic pigs with modification of the immune system that allows for xenogeneic transplants, e.g. human tissues.

Materials and Methods
The planned studies assume the use of approximately 70 transgenic pigs. On the selected transgenic pigs preclinical studies of a new treatment of cartilage injuries will be performed. Transgenic pigs were obtained using the Zinc Finger Nuclease technology (ZFN) in combination of microinjection procedure. In the established research model, cartilage of the knee joint of transgenic pigs was surgically injured. Subsequently, human mesenchymal stem cells (MSCs) were injected into the knee joint. Clinical observation was performed after the treatments.

Results and Discussion
To date, 21 transgenic animals have been obtained. After MSCs injection into the knees of transgenic pigs with surgically performed knee cartilage injuries, no clinical adverse effect were observed following the transplantation, while the regenerative effects are being investigated. Transgenesis is one of the biotechnology directions that allows obtaining animals with modified genotypes. Genetically modified animals, especially pigs are very useful preclinical trials research models for regenerative medicine. In the presented project, transgenic pigs with α 1,3-galactosyltransferase knockout are used as a research model. Acute phase rejection of the xenograft take place by humoral reaction of natural antibodies directed specifically against galactose-α1-3galactose epitopes α Gal. Epitopes α Gal occur on the surface of the vascular endothelium in most species (eg. pigs) except humans, apes and old world monkeys. In turn, humans, apes and old world monkeys have natural antibodies against epitopes α Gal. The knockout of porcine GGTA1 gene coding alfa 1,3-galactosyltransferase will reduce the immunologic reaction of graft rejection of human origin. Transgenic pig model with knockout of GGTA1 gene makes possibilities to perform preclinical trials treatments of cartilage injuries using human mesenchymal stem cells (MSCs).

Conclusions
The use in presented research of transgenic pig model is an innovative approach and so far have not been used in this type of research. The obtained preliminary results indicate that the used model is optimal for assessing procedures in treatments of knee cartilage injuries with use of human mesenchymal stem cells (MSCs)

Acknowledgments
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OPTIMIZED PROTOCOL OF ANESTHESIA IN LARGE EXPERIMENTAL MODEL OF TRANSGENIC PIGS FOR TREATMENT OF CARTILAGE INJURIES WITH NEW GENERATION BIOMATERIALS AND CELL-BASED GRAFTS *IN VIVO*

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[ENGINEERING OF BIOMATERIALS 148 (2018) 55]

Introduction
The possibility of usage of transgenic pig for experimental treatment of cartilage injuries was investigated. Anesthesia and analgesia procedures in pigs are frequently required for surgically performed research treatments due to the nature of invasive procedures that are performed. Their use as preclinical models (translational research) frequently is connected with surgical implantation of dentures, serious invasive surgery and/or creation of disease conditions [1]. Selection of an appropriate protocol which considers the physiologic effects of the pharmacologic agents for anesthesia/analgesia is an important aspect of designing an experiment. Anesthesia takes into account the abolition of consciousness by inducing deep sleep and analgesia by general and local abolition of pain sensation [2]. Too deep anesthesia results in disturbances of the respiratory system, to collapse and cardiac arrest, and to a significant impairment of blood supply and oxygenation of tissues. It is very important to maintain physiological blood pressure and physiological oxygen saturation of hemoglobin and to reduce to a minimum ischemia time and keeping a proper oxygenation of tissues [3]. For the purpose of researching new generation biomaterials using a unique research model of transgenic pigs, an original model of swine inhalation anesthesia with minimal supply of anesthetic drugs and local anesthetic agents was developed. In order to maintain the proper oxygenation of tissues anesthesiological scheme was modified, which have been used so far.

Materials and Methods
In 9 transgenic pigs, three-fold complex anesthesia was performed. In the initial stage, the pigs were quenched pharmacologically by administering the administration of azaperone 2 mg / kg (Stresnil, Janssen). In the second stage, general anesthesia was induced by intravenous administration of a 1 - 2 mg / kg xylazine and 5 - 10 mg ketamine mixture. After the abolition of consciousness, animals were intubated and inhalation anesthesia was carried out. It was used inhalation anesthesia with gas mix isofluran, nitrous oxide, oxygen and air. It was used low pressure and low flow of anesthetic gases: isofluran 0,6 – 1%, nitrous oxide 20 – 25% and high pressure of oxygen 45 – 55% with positive end-expiratory pressure (PEEP) 5 mm H_2O. EKG with ST elevation, respiratory frequency, oxygen saturation (SpO_2), hyper- or hypocapnia ($PaCO_2$) and pressure in mixed anesthetic gases was constantly monitored. Low flow was used (1,5 – 2,5 L/ minute) for mix of anesthetic gases. With such a low anesthetic gas flows, anesthesia was supplemented with local anesthesia. For this purpose, the lateral side of the knee joint was injected with 8 ml of 1% lignocaine.

Results and Discussion
During the 45-minute operation, a cartilage defect was performed on the lateral femoral condyles of 6 mm in diameter and 3 - 5 mm in depth. During surgery was not monitored disturbances in EKG, respiratory frequencies was 12/min, SpO_2 was average 98% (95 – 100%), $PaCO_2$ was average 40mmHg (30 – 45%), all in physiological range. Local pain sensation was not observed.

Conclusion
The usage of presented protocol of anesthesia allows to maintenance optimal conditions of perfusioned organs and tissues with total elimination of pain sensation throughout the operation in large animal pig model *in vivo*.

Acknowledgments
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SURFACTANT-ASSISTED FABRICATION AND EVALUATION OF MACROPOROUS CALCIUM PHOSPHATE BONE CEMENTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 56]

Introduction

An ideal bone substitute should possess many properties attributed to autologously transplanted tissue. The implant should allow rebuilding not only the bone tissue itself but also its vascularization. To make it happen, the new bone should be constantly supplied with nutrients. For this purpose, materials used in bone substitution should exhibit adequate porosity. The biomimetic approach to bone tissue engineering requires the use of specialized macroporous scaffolds. Calcium phosphates cements (CPCs) are an alternative to traditionally sintered porous ceramics for applications in bone tissue engineering.[1-3] Cement preparation does not require high-temperature processing.[4] Recent research has approached the problem of inducing macroporosity inside the bone cement without influencing its normal setting.[5-6] The aim of this study was a surfactant-assisted fabrication and preliminary evaluation of the novel macroporous chemically bonded α-TCP scaffolds. The effect of surfactants addition on cement paste foamability and phase composition of final materials was investigated. Furthermore, the chemical structure of the obtained materials was determined by vibrational spectroscopy (ATR-FT-IR).

Materials and Methods

The solid phase of obtained cements consisted of the highly reactive α-tricalcium phosphate. The initial α-TCP powder was synthesized by the wet chemical method. For the synthesis, CaOH (POCH, Poland) and 85% phosphoric(V) acid (POCH, Poland) were used, both with chemical purity. As the liquid phases 2 wt% Na_2HPO_4 solutions with 10 wt% various surfactant addition were used. A number of surfactants were proposed: Tween 20 (Polyoxyethylenesorbitan monolaurate) – TW20, Tween 80 (Polyoxyethylenesorbitan monooleate) – TW80 and Tetronic 90R4 (Ethylenediamine tetrakis(ethoxylate-block-propoxylate) tetrol) – 90R4.

Two different methods of obtaining the macroporous cements were used. One of them was mixing the powder with an already foamed surfactant solution. The second method consisted in foaming already mixed cement paste. Foaming step was performed with a domestic food mixer (BOMANN, Germany) for 30s. The control samples - without any surfactant, were obtained using the same methods. Identical molds were used for all of the materials.

Foamability [%] of the cement pastes was measured according to the equation:

$$\text{Foamability} = \frac{Vs - Vc}{Vc} \; x \; 100\%$$

where:
Vs – volume of the studied cement
Vc – volume of the control cement

Phase composition and chemical structure (XRD, D-2 Phaser, Bruker; ATR-FT-IR, Tensor 27, Bruker) of the obtained cement type materials have been studied.

Results and Discussion

Foamability of the cement pastes is shown in TABLE 1.

TABLE 1. Calculated foamability of the obtained cements in comparison with the control cement.

	TW20	TW80	90R4
Method I	150%	175%	50%
Method II	80%	40%	0%

The foamability of the cements depended on the foaming method which was used during their preparation. The first method led to higher foamability of the obtained cements. The powder X-ray diffractograms of the examined samples are presented in FIG. 1. The diffractograms of all prepared cements after setting and hardening revealed the presence of two crystalline phases: αTCP and HAp.

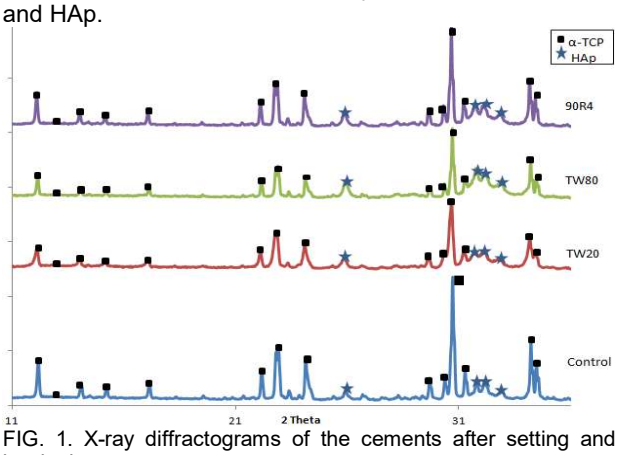

FIG. 1. X-ray diffractograms of the cements after setting and hardening.

ATR-FT-IR analysis of the obtained cements revealed that all spectra revealed the strongest bands in the regions 1200–900 and 650–500 cm^{-1} which are attributable to the vibrations of phosphate groups. The broad band from 3650 to 2600 cm^{-1} is assigned to O–H stretching (residual water). In the cements with an addition of Tweens (TW20 or TW80) C=O stretching band at 1738 cm^{-1} caused by ester structure were present. The carbon–carbon bonds of poloxamine Tetronic 90R4 appeared at 2860–2880 cm^{-1}. N-H bending in 90R4 could not be found at the same time because it coincided with the O-H stretching originated from water.

Conclusions

Macroporous calcium phosphate bone cements have been successfully obtained. The foaming method had a significant influence on their foamability. This parameter differed from 0 to 175%. Cement pastes with Tween (20 or 80) addition revealed higher foamability values than pastes with Tetronic 90R4.

The diffractograms of all studied samples revealed the presence of only two crystalline phases: αTCP and HAp.

FT-IR spectra confirmed the presence of surfactants in the obtained materials.

Macroporous, foamed cements need further research.

Acknowledgments

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HYDROXYAPATITE - CHITOSAN MATERIAL WITH SILVER NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 57]

Introduction

Materials composed of nanoparticles as well as bio-inspired composites consisting of biomimetic apatite and natural polymers such as collagen and chitosan are considered as the promising future biomaterials. Recently, the attention has been drawn to hydroxyapatite-chitosan (HA/CTS) composite, which show promise in mimicking both, the organic and inorganic components of natural bone [1,2]. Although the composite materials are the subject of many studies, a number of both cognitive and applicational, issues have not been explored yet. The role of chitosan in creating the mechanical strength of the composite still has not been explained.

The aim of this study was to obtain homogenous HA/CTS and HA/CTS/Ag composites via a simple solution-based chemical method. Special emphasis has been made on combining in the developed materials relatively good mechanical strength with the desired microstructure. The influence of the addition of 0.1wt% silver nanoparticles on physicochemical properties of materials has been examined.

Materials and Methods

The new hydroxyapatite-chitosan (HA/CTS and HA/CTS/Ag) materials were synthesized via modified wet chemical method using $Ca(OH)_2$ (≥99.5%, Merck), H_3PO_4 (85.0%, POCH) as the sources of calcium and phosphorus ions, respectively. Medium molecular weight chitosan (around 100,000 kDa, DD ≥ 75.0%, viscosity 200–800 cups, Sigma-Aldrich) was applied as the organic component. The procedure, by which materials were prepared, has benn described previously [3]. After decanting HAp/CTS slurry, suspension of 0.1wt% of silver nanoparticles (US Research Nanomaterials, NC., USA) was added. The crystalline phases of the obtained materials were analyzed by powder X-ray diffraction with CuKα radiation (D2 Phaser, Bruker) in the 2θ range of 10°–60° at a scanning speed of 1°/min. The scanning electron microscope (SEM - Nova 200 NanoSEM, FEI Company) equipped with X-ray dispersive spectroscopy (EDS) was used to determine crystal morphology and chemical elemental composition in microareas of the samples. For the compressive strength testing, the cylindrical samples (6mm in diameter and 12mm high) were prepared from the synthesized filter cake and stored at 37 °C for 1week. The compressive strength was measured at a crosshead displacement rate of 1.0 mm·min−1 using universal testing machine Instron 3345.

Results and Discussion

During the synthesis of HA/CTS hybrid material via the modified solution-based method the electrostatic complexes between positively charged, protonated amine groups of chitosan and the negative phosphate species (HPO_4^{2-} and $H_2PO_4^-$) were formed. X-ray diffraction analysis revealed that the prepared samples consisted of only one crystalline phase – i.e. hydroxyapatite (FIG. 1). The characteristic reflections for non-stoichiometric apatitic structure of low crystallinity degree were detected in the patterns of studied materials.

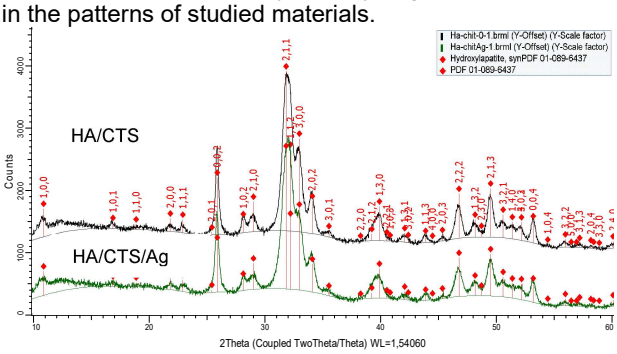

FIG. 1. X-ray patterns of HA/CTS and HA/CTS/Ag materials.

SEM observations demonstrated that the applied preparation method allowed obtaining materials with homogeneous microstructure (FIG. 2). The observed changes in the surface morphology were connected with the addition of A nanoparticles to the HAp/CTS materials.

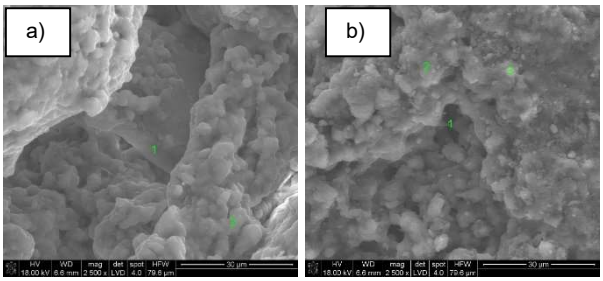

FIG. 2. Microstructure of material a) HAp/CTS and b) HAp/CTS/Ag.

Mechanical strength is usually the imperative criterion during the selection of bone substitutes for tissue engineering. Materials HAp/CTS and HA/CTS/Ag possessed compressive strength 22.4±7.4 MPa and 22,1.1±9.1 MPa, respectively. The addition of 0.1 wt.% of silver nanoparticles to HA/CTS material did not influence significantly on compressive strength.

Conclusions

Results of our studies have shown that it is possible to introduce chitosan and silver nanoparticles into the structure of hydroxyapatite via the wet chemical synthesis. This method allows us to obtain the ceramic-polymer composite with an interesting, promising mechanical and biological properties. X-ray diffraction analysis, revealed the presence of only one crystalline phase – i.e. hydroxyapatite in HA/CTS and HA/CTS/Ag materials. Introduction of chitosan and silver nanoparticles during the synthesis did not influence the distribution of hydroxyapatite. SEM observations demonstrated that the preparation method, allowed obtaining composite materials with homogeneous microstructure. The compressive strength of obtained materials was equal 22MPa. The obtained HA/CTS/Ag materials due to their interesting properties could be potentially used as a solid phase of bone cements.

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ANTIBACTERIAL ACTIVITY ASSESSMENT OF BIOACTIVE MODIFIERS FOR BONE CEMENTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 58]

Introduction

Active biomaterials have an important role in modern medicine, because their using can reduce the risk of postoperative complications.

Apart from its main function, i.e. bone filling and fracture stabilizing, bone cement can also be applied as a carrier of bioactive agents. Then due to the releasing of the substances it enables: combating bacteria, protecting against microorganisms and treating local infection [1-3].

In this work, analysis of the bactericidal effectiveness of selected bioactive agents used as cement modifiers was performed.

Materials and Methods

The research was carried out on commercially available PMMA bone cement – Cemex (Tecres, Italy) and following bioactive additives were used:

- antibiotics /1.5w/w%/:
  - gentamicin (Sigma Aldrich, Germany);
  - ciprofloxacin (Sigma Aldrich, Germany);
- nanometals /5w/w%/:
  - silver nanoparticles – 50 nm (MkNano, Canada)
  - copper nanoparticles – 30 nm (MkNano, Canada);
- chitosan medium molecular weight particles /3w/w%/ (Sigma Aldrich, Germany).

Preparation of bioactive bone cements was conducted in accordance with previous study [1]. The additives were added to the powder and hand-mixing. Next the bone cements were prepared following the procedure by the manufacturer's recommendation. Then this obtained paste was placed into molds to ensure the required shape and allowed to cure for 1 hour in ambient conditions.

To determine the bactericidal properties of modified bone cements, the bacterial growth inhibition zone tests were performed. For research three clinical isolated bacterial strains were taken: *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (supplied by Specialist Hospital in Kościerzyna, Poland).

Results and Discussion

The porous structure of the bone cement enables the deposition of bioactive agents in it. Then, as a result of the flow and circulation of body fluids, the substance particles are gradually released into the environment [1]. Therefore, the porosity and the structure of cement have the greatest impact on the effectiveness of its bioactive ability, but also are the following features are important: the amount of substances used, its form and particles size [1,3].

Generally, the gold standard for commonly used bioactive biomaterials is the use of antibiotic additives. However, due to the growing problem of bacteria resistance, mutations and the formation of biofilm this method becomes problematic [2]. Therefore, currently other bioactive substances are sought. The following features are expected for this potential additives for biomaterials: a broad spectrum of activity, a lack of resistance, an ability to combat biofilm and a long therapeutic period [2]. On the other hand, it is important that these modifications do not interfere with biomechanical properties of biomaterial [1].

Nowadays, the only method for commercially available cement is the addition of antibiotics. However, other solutions are constantly searched and tested [1-3].

In this work, five types of bioactive agents used for modification of bone cement were studied:

0 – unmodified bone cement,

1 – bone cement modified with nanosilver,

2 – bone cement modified with nanocopper,

3 – bone cement modified with ciprofloxacin,

4 – bone cement modified with gentamicin,

5 – bone cement modified with chitosan.

A bacterial growth inhibition tests for common strains of bacteria were carried out (FIG. 1).

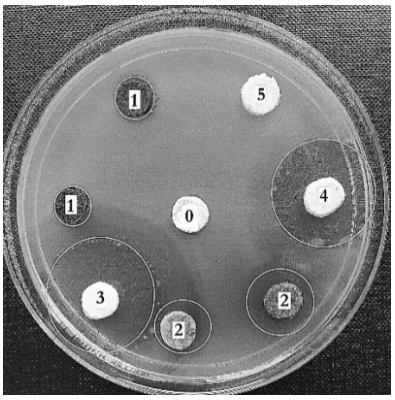

FIG. 1. Comparison of the bacterial growth inhibition zone for the tested specimens after 72 h; circles – visible growth inhibition zone

The bactericidal effectiveness of bone cements modified with antibiotics and nanometals has been confirmed (FIG. 1). The largest zone of bacterial inhibition was observed for the antibiotic - ciprofloxacin. However, the smallest for nanosilver. Moreover, the lack of efficiency was found for chitosan. The experiment lasted 7 days and both antibiotic-loaded cements and cement modified with nanometals maintained their activity during this period of time.

Conclusions

Modified bone cements can be used as carriers of bioactive agents and fight locally the bacteria. Hence, they can be used to treat infections or its prevention.

Acknowledgments

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SYNTHESIS AND CHARACTERIZATION OF POLYURETHANE-BASED BIOMATERIALS MODIFIED WITH CHITOSAN AND HYDROXYAPATITE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 59]

Introduction

The tissue engineering offers new opportunities in regeneration of the damaged tissue in a human body when natural processes are impossible. One way to dissolve this problem is an introduction of a scaffold to give the cells the forming ability of new tissue [1,2]. Proper selection of physical and chemical properties of the scaffold material enables attachment and proliferation of cells. The polyurethanes are known to exhibit biodegradable, bioresorbable and non-toxic properties [3]. Polyurethanes are polymers composed of hard segments derived from a diisocyanates and chain extender, and soft segments formed from polyether or polyester. This type of microphase-separated structure proved different properties of polyurethanes depending on the ratio of soft and hard segments [4]. An alternative approach to achieve a bioactive polymer scaffold with appropriate mechanical quality is via incorporation of ceramics like hydroxyapatite (HAp) [5]. Along with biocompatible nature and non-toxic character of chitosan, that polysaccharide is applied as a chain extender/curing agent for PU. Chitosan has a significant impact on the enhancing thermal properties and biocompatibility of PU elastomers [6]

The aim of the research was to obtain polyurethanes, which may be used as bone cement and will show lower thermal effect during crosslinking, due to the introduced phase change materials (PCM) consisting of poly(ethylene glycol) and chitosan. Moreover, the introduction of hydroxyapatite to polyurethanesacharides was investigated towards the bioactivity of composite material.

Materials and Methods

The biodegradable polyurethanesaccharides containing phase change materials were obtained using bulk polymerization method. The prepolymerization of 1,6-hexamethylene diisocyanate (HDI) and poly(ethylene glycol) 2000 (PEG-2000) were carried out in a nitrogen atmosphere with dibutyltin dilaurate (DBTDL) as a catalyst. The obtained prepolymer was cured with 1,4-butanediol (BDO) and chitosan in different molar ratio. Hydroxyapatite was introduced to enhance biocompatibility and osteoconductivity. Differential scanning calorimetry (DSC), themogravimetric analysis (TG) and dynamic mechanical analysis (DMA) methods have been used to investigate the thermal properties of the material. The presence of hydrogen bonds was confirmed by Fourier-transform infrared spectroscopy (FTIR). The results of polyurethane hydrolytic degradation in a phosphate-buffered saline (PBS) and Ringer solution were presented. The formation of apatites on the polyurethane surface after incubation in simulated body fluid (SBF) was confirmed by scanning electron microscopy (SEM).

Results and Discussion

The influence of different molar ratio of chitosan and hydroxyapatite was analyzed. The thermal properties of the polyurethane were investigated using differential scanning calorimetry (DSC). The glass transition of soft segments has been found in the range of -47°C and –57°C. With the incorporation of chitosan to PU a slight decrease of melting temperature (T_m) (from 39 to 33°C) and the heat of phase transition (from 56 to 53 J/g) for soft segments from polyether component was observed. The TGA analysis showed multi-step decomposition of polyurethanes modified with chitosan and hydroxyapatite. Importantly, the initial decomposition temperature increased with a higher concentration of chitosan. The results from the FTIR and SEM studies prove the existence of chitosan and hydroxyapatite in polyurethane structure. FTIR spectrum of obtained polyurethanesaccharides showed the absorption bands at 1689 cm^{-1}(-C=O stretching) and 3310 cm^{-1} (-NH stretching) derived from urethane group in obtained polyurethanes.

Conclusions

Polyurethanesaccharides modified hydroxyapatite and chitosan were synthesized in a two-step polymerization method. It has been showed that chitosan as a chain extender improves the thermal stability of polyurethanes. The obtained materials have a potential for application as a replacement of acrylate bone cements.

Acknowledgments

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NANOENGINEERED HYBRID SILICA/ORGANIC NANOPARTICLES AND IONIZED GASES FOR BONE REGENERATION THROUGH SMART SCAFFOLDS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 60]

Introduction

The use of degradable scaffolds promoting stem cells adhesion and proliferation is one of the most promising approaches today in tissue regeneration. However, this approach is still facing many challenges such as cost, mechanical properties and shaping of the scaffolds, revascularization of tissues, drug delivery on long durations and the ability of the scaffolds to promote efficiently the cells adhesion and proliferation. A way to solve a part of these challenges is to embed or attach specifically engineered nanoparticles into these scaffolds and to treat these scaffolds by ionized gases. Silica-based mesoporous nanoparticles are particularly well suited for the bone regeneration due to their slower dissolution rate in physiological media, their high drug loading capacity, their high ability to be functionalized by biodegradable coatings and their biocompatibility. In this work, two approaches are studied to improve the scaffold properties for bone regeneration through mesoporous silica nanoparticles and ionized gas. The first one is based on silica nanoparticles functionalized with biocompatible coatings made of lipids or/and polysaccharides. By this way, active molecules are well protected during their attachment to the scaffolds or during sterilization processes. And active molecules release can be better controlled. In the second approach, ionized gas is used to allow the functionalization of the scaffolds for nanoparticles attachment, sterilization and promotion of cell attachment and proliferation. The interaction of silica nanoparticles and ionized gas with scaffolds will be discussed. As a first result, a good dispersion of these nanoparticles has been obtained in polymeric scaffolds as monitored with the fluorescent probes placed inside the nanoparticles.

Materials and Methods

Mesoporous silica nanoparticles with a pore size around 10 nm were synthesized using a sol-gel approach in alkaline conditions [1]. A surfactant, CTAB, a swelling agent and a base, TEA, are added in a hydro alcoholic solution at room temperature and stirred. Then, the temperature is increased to 60°C and the silica precursor, TEOS, is added drop-by-drop in this mixture. The solution is stirred during 2h at 60°C. APTES is added 20' after TEOS in order to get amine chemical groups on the external part of the silica nanoparticles. These inorganic nanoparticles are then coated with a lipid bilayer and a calcium alginate coating by using cycles of mixing, ultrasonication and centrifugation [2], or with a polysaccharide multilayer by using the layer-by-layer approach [3]. Finally, these hybrid nanocarriers are embedded in a polymer scaffold made of polycaprolactone containing polyurethane units [4]. For plasma activation of the scaffold, a pulsed radio-frequency plasma jet Neoplas KinPen 11 working at atmospheric pressure was applied either directly on the scaffold in air or with a film of water on top [5].

Results and Discussion

The pore size was controlled by adding a swelling agent, ethyl acetate, in the hydroalcoholic solution with the other reactants. The ethyl acetate molecules are swelling the micelles formed with the surfactant, up to 10-15 nm, leading to pores of similar size after cleaning/dissolution processes, without changing the main characteristics of the nanocarriers synthesized without swelling agent, including the particles diameter (50-80 nm) as shown on FIG. 1. By this way, proteins can be encapsulated and protected, after adding an organic coating, inside these nanocarriers before to be embedded in the scaffold.

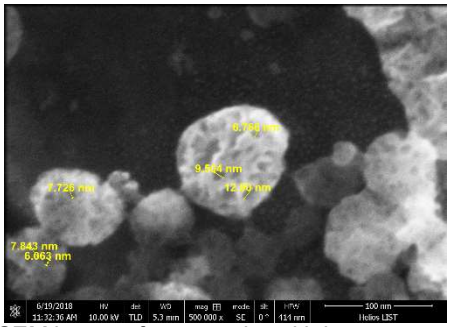

FIG. 1. SEM image of nanocarrier with large pores.

The different organic coatings deposited on the silica core nanoparticles were shown to avoid active molecule leakage and provide a good biocompatibility to the hybrid nanocarriers. Then, two approaches are targeted to attach the nanocarriers to the polymeric scaffold. In the first one, the scaffold has to be activated by the ionized gas jet in order to generate chemical groups able to react with the organic coating of the nanocarriers. It is shown that both in water and air, O-containing groups are formed in a range of 1% in water to few percent in air by applying the plasma jet for a few minutes on the scaffold. These functional groups will allow to attach the hybrid nanocarriers more easily to the scaffolds. In the second approach, the hybrid nanocarriers are directly suspended in the emulsion used to generate the porous scaffold. By this way, a homogenous dispersion of the hybrid nanocarriers was obtained.

Conclusions

Mesoporous-based silica nanoparticles were synthesized and functionalized in order to be able to protect and deliver proteins for bone regeneration through a polymeric scaffold. Ionized gas was used to prepare scaffolds for attachment of the hybrid nanocarriers. The organic coatings allowed to protect the payload and to disperse the nanoparticles inside the scaffolds. Next steps will focus on up-scaling of nanocarriers and biological tests.

Acknowledgments

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CARBON NANOTUBE-REINFORCED HYDROGELS FOR BONE TISSUE REGENERATION

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[Engineering of Biomaterials 148 (2018) 61]

Introduction
Whey protein (WPI), a protein derived from dairy sources, and a by-product when producing foods such as Greek yoghurt, has attracted attention for potential applications in tissue engineering. The primary component of WPI is β-lactoglobulin, which is known to promote cell proliferation. Additionally, a recent study [1] demonstrated that cells can adhere, differentiate, and proliferate on WPI hydrogels – which can be fabricated and sterilised in a singular step, by autoclaving. The ability to incorporate particles into hydrogels provides them with further potential advantages for tissue regeneration, including Mechanical reinforcement, antibacterial properties, and further promotion of bone-forming cell proliferation. The inclusion of carbon nanotubes (CNTs) into WPI hydrogels may provide such advantages, as the unique structural, mechanical, electrical and chemical properties of CNTs are already well documented. In this study, CNTs were incorporated into WPI hydrogels, and both the physiochemical and biological properties were evaluated.

Materials and Methods
40% WPI solution (in ddH_2O), was mixed with 0 to 40% CNTs by alternate sonication and vortexing, in Eppendorf tubes. Hydrogels were then simultaneously fabricated and sterilised by autoclaving.
The physical properties of hydrogels were examined using SEM, FT-IR and mechanical testing, while antibacterial tests on *S.aureus* and cell culture was also performed. MG63 osteoblast -like cells were seeded onto hydrogels, before the morphology, proliferation and adhesion of cells were observed at intervals of 1, 4 and 7 days using fluorescent microscopy, coupled with the MTS assay.
All statistical significance was evaluated using one-way ANOVA on SPSS.

Results and Discussion
SEM (Fig. 1) illustrated the successful incorporation of CNTs into the WPI hydrogels. This interaction of WPI with CNTs was further supported by FT-IR (data not shown). Mechanical testing also displayed that the incorporation of CNTs coincided with an elevated Young's modulus, but a decrease in compressive strength (data not shown). Antibacterial testing also displayed a reduction in bacterial growth when CNTs are present. Importantly, even 20% WPI-CNT samples displayed MG-63 cell growth, even after 7 days. Therefore, WPI-CNT hydrogels show promise as a material for future research into bone tissue engineering.

Conclusions
WPI-CNT hydrogels exhibited antibacterial activity against S.aureus, and growth of MG63 cells was not apparently worse on WPI-CNT hydrogels. Finally, the addition of CNTs resulted in stiffer hydrogels with lower compressive strength.

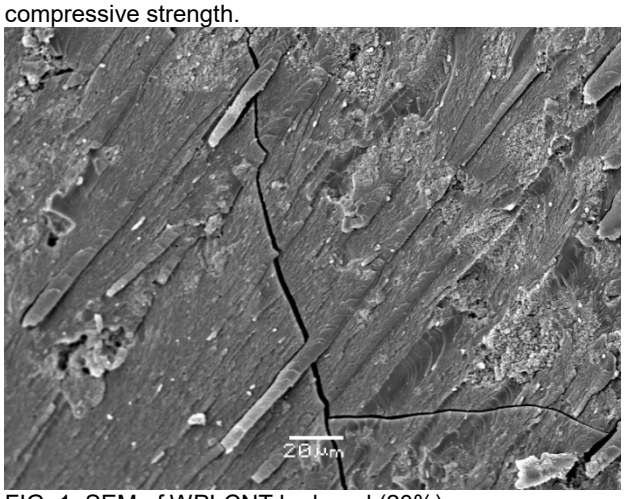

FIG. 1. SEM of WPI-CNT hydrogel (20%).

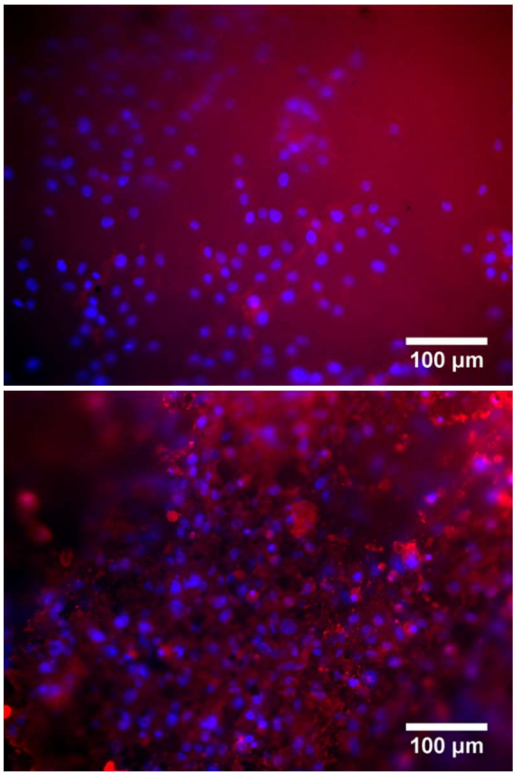

FIG. 2. Fluorescent microscopy of WPI control (left) and WPI-20%CNT (right) after cell culture with MG-63 cells (images after 7 days, red- Texas red, Blue- Hoescht stain).

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BIOMIMETICALLY MINERALIZED GELATIN HYDROGELS PRODUCED BY NOVEL BETA-RADIATION CROSSLINKING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 62]

Introduction

Hydrogels have numerous potential advantages in tissue engineering. One of these is the ease of incorporation of bioactive substances such as enzymes. Gelatin is a promising biomaterial, being inexpensive, biocompatible, non-immunogenic and biodegradable. However, gelatin hydrogels lose integrity at body temperature without crosslinking. In this study, gelatin hydrogels were crosslinked using electron beam irradiation [1]. This allows simultaneous crosslinking and sterilization while avoiding use of cytotoxic chemical crosslinkers (e.g. glutaraldehyde). In order to improve hydrogels for bone tissue regeneration, mineralization with calcium phosphate (CaP) is desirable. Advantages include bioactivity (formation of a chemical bond with adjacent bone following implantation) and mechanical reinforcement. In this study, the enzyme Alkaline Phosphatase (ALP) was incorporated into electron beam-crosslinked gelatin hydrogels to induce their mineralization with CaP [2]. Subsequently, both physiochemical and biological properties were evaluated.

Materials and Methods

Hydrogels were produced by Riedel publications – ALP was added (0, 1.25 or 2.5 mg/ml) to an 8mg/ml solution of type I gelatin in ddH_2O. This solution swelled for 1 hour at room temperature, and was subsequently heated to 37°C, poured into moulds, and allowed to polymerise for 12 hours at 6°C.
Samples were irradiated, under cooling, by a linear electron accelerator.
Samples were subsequently incubated in either ddH_2O or 0.1 M CaGP for 14 days.
Mineralization was assessed by SEM, Raman, FTIR and ICP-OES
5000 MG63 osteoblast-like cells were seeded onto hydrogels, and at intervals of 1, 4 and 7 days, the morphology, proliferation and adhesion of cells on hydrogels were observed.
Mechanical testing was also performed.
All statistical significance was evaluated using one-way ANOVA on SPSS software.

Results and Discussion

SEM (FIG. 1.) illustrated the formation of CaP deposits in hydrogels containing ALP, which was further supported by FT-IR, Raman, and ICP-OES (data not shown). Mineralisation also coincided with an elevated compressive modulus (data not shown).
Cell culture (FIG. 2 and 3) demonstrated that cells were still alive, and metabolically active, at day 7 of cell culture, even at the highest ALP concentrations.
Electron irradiation of gelatin hydrogels, coupled with enzymatic mineralisation provided a successfully mineralised, stable hydrogel, which maintained biological activity. Therefore, the present method is promising for future research into bone tissue regeneration materials.

Conclusions

Gelatin hydrogels enriched with ALP and crosslinked by electron beam irradiation were successfully mineralized with CaP, leading to mechanical reinforcement. Electron beam-crosslinked hydrogels, both mineralized and unmineralized, supported MG63 cell adhesion and proliferation.

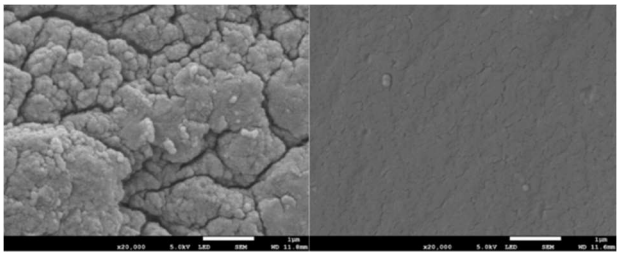

FIG. 1. SEM of Gelatin hydrogels after mineralisation (14d). Left: 2.5mg/ml ALP in CaGP. Right: 0mg/ml ALP in ddH_2O.

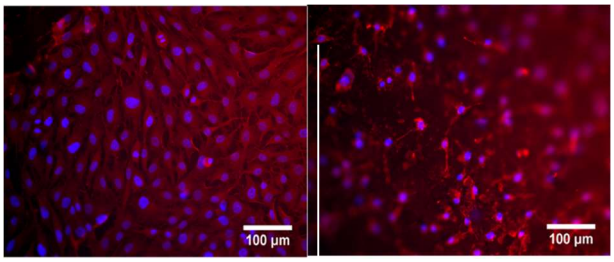

FIG. 2. Fluorescent microscopy of 0 mg/ml ALP in H_20 day 7 (left) and 2.5 mg/ml ALP in CaGP (right) stained with Texas red (red) and Hoechst (blue).

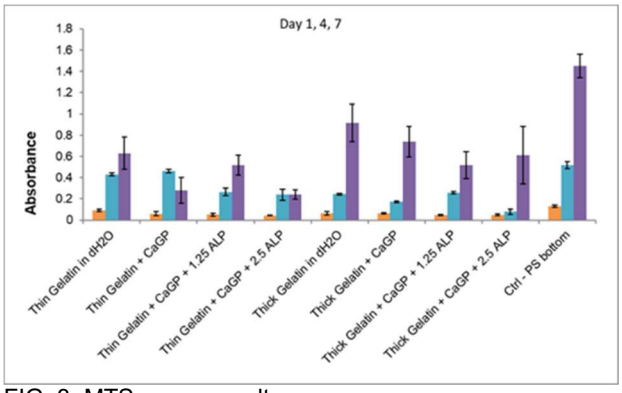

FIG. 3. MTS assay results.

Acknowledgments

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FIBROUS STRUCTURES BASED OF NATURAL POLYMERS FOR TISSUE ENGINEERING APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 63]

Introduction

One of the directions of tissue engineering is the search for new materials based on natural origin polymers. Nowadays, derivatives of chitin and hyaluronic acid, collagen, proteins or alginates are of great importance in the preparation of materials for various types of scaffolds [1-2]. The use of these polymers to obtain hybrid materials based on both biopolymers and other bioactive substances creates opportunities for faster tissue regeneration and thus increases the effectiveness of the therapy undertaken. For this type of materials, it is important to use biocompatible substances, also of appropriate structure, or even a personalized, hierarchical model that will allow the proper interaction of the biomaterial with natural tissues. The aim of this work is to present examples of the use of fibrous structures for the production of hybrid materials for regeneration of bone tissue.

Materials and Methods

In the work fibres made at the Lodz University of Technology (LUT) were used. The developed layered systems (mass per unit area of ca.100g/m^2, FIG. 1) consist of one inner layer of two kind of modified calcium alginate (CA) fibres (with nanoparticles of hydroxyapatite, (HAp) and Fe_3O_4 introduced into their structure, CA+HAp/CA+Fe_3O_4) and two outer layers of poly(lactic-co-glycolic acid (PGLA) nanofibres. The inner layer was produced using the needled nonwoven method and different proportions of both modified fibres. The nanofibres forming the outer layers were applied by electrospinning method. For selected versions of the systems a physical modification with the RGD peptide has been undertaken. Such solution supports cell regeneration and increases cell adhesion by integrin activation.

The materials were examined using scanning electron microscopy (SEM) and Raman spectroscopy techniques.

The stability of these hybrid fibrous materials was tested (*in vitro*) by incubation in two media: phosphate buffer (PBS) and water (temperature 37°C, 5% CO_2). Degradation tests were carried out for 36 days.

Results and Discussion

The chemical structure of the modified fibres containing bioactive additives was confirmed using Raman spectroscopy. The spectral analysis results in the presence of characteristic peaks coming from the vibrational band of carboxylane group, M and G blocks of alginate. The resulting differences between the spectra are mainly related to the presence of modifying compounds. Obtained layered materials are characterized by the anisotropy of the distribution of elementary fibres in the material structure. This effect is extremely important from the point of view of the application of this type of material. In the case of material degradation studies, the applied test procedure allowed to establish a certain tendency regarding the durability of nanocomposite nonwovens based on calcium alginate; the more complex multi-electrolyte fluid (such as a phosphate buffer) the shorter the degradation time. In the case of PBS the inner hybrid nonwoven was disintegrated after 36 days. The medium after the degradation test contains a deposit composed mainly of nanocomposite CA fibres and a thin fibrous layer from outer PGLA nonwovens.

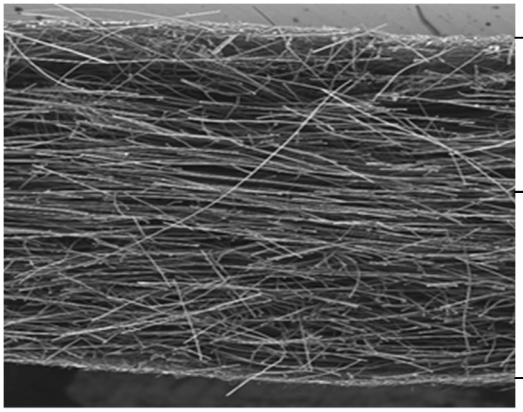

FIG. 1. SEM image of the layered system sample; 1 – inner layer of CA+HAp/CA+Fe_3O_4, 2 – outer layers of PGLA.

Conclusions

The SEM and Raman spectroscopy studies confirmed the layering formation of the fibrous systems as well as their chemical structures and various content of the used modified fibres. Degradation studies in the immersion media have shown different degradation effect of developed layered systems over time. This creates the possibility of modelling fibrous structures with a controlled degradation process.

Acknowledgments

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BIOMATERIALS IN CORRECTION OF INGROWN NAILS – OVERVIEW OF CURRENT ISSUES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 64]

Introduction

The problem of ingrown nails (Latin: Unguis incarnatus / Onychocryptosis) is known in scientific and medical literature [1,2] as this problem cause incapacitation and real pain for the patient and impossibility of normal functioning. However, apart from the surgical methods, there are many ones that are commonly used by the podiatry surgeries. The methods used in them - although also in medical facilities - are based mainly on the use of so-called "temporary" implants for correction of the nail plate [3]. A method involving such a practice is called Orthonyxia, which consists of implantation of a small metal (or other material) brace or plate onto the dorsum of the nail [4]. The method is characterized by high efficacy and non-invasive compared to classical surgical methods, which translates into better tolerance for patients and not excluding them from everyday duties [5]. However, to date, no work has been developed covering the entire subject in terms of materials. This review work is an initiation of research and ordering of issues.

Materials and Methods

In the discussed issue, we can distinguish two main areas - the area related to the parameters associated with the nail plate and the area associated with the biomaterial parameters used for Orthonyxia treatments. In the first area should be mentioned the basic dimensions of the nail plate as thickness, length, width, surface, and other more complex parameters such as the elasticity of the distal edge (border) of the nail, the length of protrusion beyond the nail bed, angle of curvature of the growth edge, surface structure and depth of possible ingrown nail plate into nail walls. Next, parameters of the surface topography and nail plate associated with its deformation such as transversal deformation and longitudinal deformation.

The tool appropriate to complete the listed parameters can be scientific device Nail StrainStress Meter NM 100 by Courage + Khazaka electronic GmbH.

On the other hand, we have a number of parameters related to the materials used. Widely used materials in Orthonyxia are braces plastic, plastic-silicone, fiberglass, gold (aesthetic area), steel and metal alloys from which the appropriate braces are made. The last of these, metal alloys, are among the most popular ones used in the last decade. Especially memory shape alloys used in orthodontics.

The problem, as in the previous area related to the nail plate itself, is that no mechanical and other parameters related to the forces released by the materials used and their effect on the biological environment of the application site, which is often a wound environment, are determined.

Therefore, it seems to be strategic to determine the basic operating parameters of the used metallic biomaterials with the shape memory as the chemical and physical structure of the alloy, electron activity, possible functional groups on the alloy surface, mechanical properties (Weibull module, Young's modulus, fracture energy, stiffness, hardness, elasticity, coefficient of friction), and susceptibility to corrosion. Also parameters related to surface topography of the alloy like roughness and porosity [6].

Further, issues related to the biocompatibility of the material should be considered, whereas materials used in the oral environment may behave completely differently than in the environment in which the foot works, often with the injured wound. It will also be important to determine the susceptibility to cell deposition and allergenic (oxidation of metal ions) or metallosis potential.

The above data in part may be completed from manufacturers of specific alloys. Others will have to be completed by performing tests in a suitable biomaterial laboratory.

This will allow to determine specific vectors of acting forces generated by the shape of a brace made of a particular selected biomaterial, on the nail plate in a given specific case of disease.

Results and Discussion

To determine whether Orthonyxia is an acceptable and effective alternative to surgery it is necessary to carry out research to determine how nail parameters, nail structure and duration of problem are in relation to the acting deformation force triggered by the specific biomaterial used for implantation.

Polish biomaterials/biomedical experiment, as part of world Orthonyxia studies, proposes a solution based on known problems to measure and collect all mentioned above necessary parameters to prepare a book of recommendations for all those professionaly involved in the area of Ortonyxia. We planned for the implementation of a broad clinical trial of >120 people, which aims to demonstrate effectiveness of collected and developed data.

Conclusions

There is a strong need to organize the whole of the Orthonyxia issues in terms of the applied biomaterials and their properties, and effects on correction of ingrown toenails. Mechanical, chemical and biological properties.

This will allow for more precise selection of biomaterials for specific cases of disease in relation to a person, and thus a higher efficiency with fewer complications and suffering on the part of the patient. Increasing efficiency should also affect the economic availability of the method, according to the premise that the less inefficient treatments, the lower the cost of treatment.

Acknowledgments

Acknowledgments for prof. Elżbieta Pamuła from Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Science and Technology in Krakow, Poland.

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MICRO- AND MACROSCOPIC PROPERTIES OF CHITOSAN COLLOIDAL SOLUTIONS INVESTIGATED BY RHEOLOGICAL METHODS COUPLED WITH THE SMALL ANGLE LIGHT SCATTERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 65]

Introduction

Chitosan is an amino-polysaccharide obtained by deacetylation of chitin. Chitosan, as a natural biopolymer, a substance with biocompatible and biodegradable properties [1], has found a wide application in medicine, food and cosmetics industries as well as in wastewater treatment processes [2].

In this work, we aimed to study the microstructural and macroscopic properties of chitosan colloidal systems. An analysis of properties of colloidal chitosan solutions using rheological methods combined with a simultaneous small angle light scattering (SALS) measurements was conducted.

Materials and Methods

The measurements were carried out using the Anton Paar Physica MCR 502 rheometer equipped with an integrated SALS optical analysis system. The study concerns chitosan colloidal system with addition of disodium β-glycerophosphate [3].

To determine viscous properties of the medium, the sample was subjected to rotational shear for 60 s at subsequent fixed shear rates of 1...500 s^{-1}, repeating the procedure toward decreasing shear rate values. The process of thermo-induced gelation was carried out under oscillatory shear at fixed strain amplitude γ = 1%, and angular frequency ω = 5 rad/s. The samples were heated from 5°C (storage temperature) to 80°C, maintaining constant heating rate of 1 K/min. The light source was 10 mW LED diode laser operating at a wavelength of 658 nm.

Results and Discussion

The investigated chitosan system reveal a shear thinning behaviour. Simultaneously with a viscosity decreases, a change in the shape of the scattering pattern from circular to elliptical was observed. This change represents orientations and deformations of the polymer domains. Based on the eigenvalues of the second-order tensor of the intensity distribution, the anisotropy was determined to quantify a deformation phenomenon.

The gelation temperature, radius of gyration, and conformation of chitosan molecules were determined based on the oscillatory shear and non-isothermal measurements combined with recording of SALS data. The rheological (crossover point of dynamic moduli and Fredrickson-Larson method) and light scattering (Zimm plot) approaches were used to determine values of phase transition temperatures. Quantification of the chitosan molecules size was achieved by applying the Debye function in order to determine the radius of gyration. Changes in the radius of gyration as a function of temperature for all tested concentrations of colloidal chitosan solutions are shown in FIG. 1.

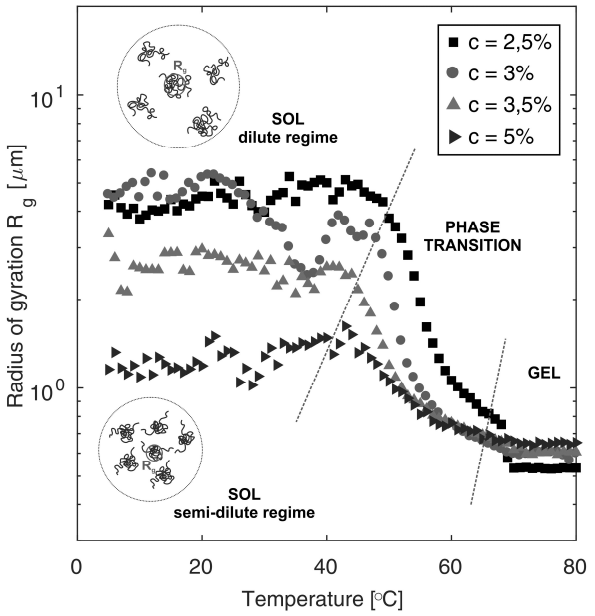

FIG. 1. Changes in the radius of gyration R_g as a function of temperature and the schematic presentation of chains conformations depending on concentration regime.

Conclusions

The conducted studies have shown that in colloidal chitosan solutions, the polymer domains occur in the form of Gaussian coils. Based on rotational shear measurements, it was found that chitosan solution reveals two different behaviours delimited by the critical value of the shear rate.

It was found that with an increase of chitosan concentration, the molecule size decreases. The above statement indicates that in more concentrated solutions, the chitosan coils are more tangled and smaller but occur in a larger number than in the diluted solutions.

Acknowledgments

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APPLICATION OF POLYMER- AND GRAPHENE- BASED MATERIALS IN BIOMEDICAL RESEARCH

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[Engineering of Biomaterials 148 (2018) 66]

Introduction

Regenerative medicine, combining biomaterials and stem cells constitute an innovative field in biomedical research. Mesenchymal stem cells (MSCs) represent a class of adult multipotent stem cells. They possesses the ability to differentiate into cells of mesodermal origin, including osteoblasts, chondrocytes and adipocytes. MSCs are characterized by e.g. high proliferative capacity and paracrine activity. Moreover, MSCs are easy to harvest from different sources, demonstrate low immunogenicity and may be applied in autologus and allogenic transplantations. All these features makes MSCs as one of the most promising stem cells type for regenerative medicine.

Nowadays, innovative approaches which may improve regeneration process, are a wide of interest. Growing evidence indicate, that MSCs in combination with (bio)materials constitute novel perspectives for tissue engineering and biomedical applications. Thus, natural polymers and graphene-based substrates constitute promising platforms for stem cells applications.

Materials and Methods

In this study, we develop novel concept of stem cell utilization in association with biocompatible composites. We used graphene-oxide (GO), reduced graphene-oxide (rGO) and natural polymers, such as chitosan and alginian as scaffolds for stem cell- related applications.

MSCs were isolated from human umbilical cord Wharton's jelly (hUC-MSCs) using an explant method. Cells were cultured in DMEM/F12 medium supplemented with 10% FBS in an incubator chamber at 37^0C, 5% CO_2 and 95% humidity. GO, rGO and polymer- based matrices (that were modified by graphene and hydroxyapatite) were tested as culture surfaces dedicated for hUC-MSCs. Moreover, the influence of GO and rGO-based scaffolds on chondrogenic and osteogenic differentiation capacity of hUC-MSCs was evaluated in *in vitro* condition.

Results and Discussion

Obtained results revealed that graphene- and polymer-based substrates constitute non-toxic surfaces for hUC-MSCs. We observed morphological differences of hUC-MSCs cultured on both types of tested scaffolds as compared to cells cultured on control plate (tissue culture polystyrene surface, TCPS). Moreover, the analysis indicated that decrease in proliferation capacity and metabolic activity of hUC-MSCs, cultured on modified surfaces, may be related with their high differentiation potential. Quantitative analysis of gene expression revealed these observations. Our results shown that graphene-based scaffolds may enhance hUC-MSCs differentiation toward chondrogenic and osteogenic cells *in vitro.* Thus, these data may suggest, that analyzed scaffolds exhibit a potential applicability as novel, safe and biocompatible materials for utilization in regenerative medicine.

Conclusions

Stem cells and biomaterials constitute an implication for novel grafts that may be used in tissue regeneration. Obtained results indicate positive effect of graphene- and polymer- based scaffolds on functional features of hUC-MSCs. However, further studies are required to analyze these phenomenon.

Acknowledgments

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HOW TO PREPARE BACTERIA WITH ADSORBED NANOPARTICLES FOR SEM AND TEM OBSERVATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 67]

Introduction

Growing applications of nanoparticles (NPs) lead to their accumulation in the environment. The majority of scientific reports reveal their negative impacts on aquatic organisms, invertebrates, mammals, and humans. There is a need to identify and quantify the parameters governing the NPs interaction with the biological surfaces such as bacterial cell walls. Most of the studies are focused on the interaction between NPs and bacteria, leading to an apoptotic disintegration of the bacterial cells (cells death), but there is a need to optimize a biological model of this interaction and propose reliable methods for electron microscopy observations. We believe that such knowledge may be used for further investigation of microorganism-based effective systems, not only for the capture of NPs from the environment but also for the development of new functionalities. The aim of this study was to optimize the procedures for preparing bacteria coated with NPs for SEM/TEM observations.

Materials and Methods

The investigated bacteria (*Pseudomonas putida* ATCC 31483) were cultured on a glass/ITO substrate and then prepared in two different protocols: (a) the bacteria cultures were directly prepared on glass/ITO substrate and after the 15 min contact with the AuNPs suspension and simply dried in air; (b) the bacteria cultures were fixed for the SEM observations according to the protocol [1,2]. Briefly, samples on a glass/ITO substrate were fixed in 3 % buffered glutaraldehyde for 24 h, and then carefully washed two times with Dulbecco's Phosphate Buffered Saline (DPBS). They were next dehydrated in the water-alcohol solutions with gradually increasing ethanol concentration (50, 60, 70, 80, 90, 96 and 100%) for 10 min each. Finally, the samples were dried using hexamethyldisilazane (HMDS). The prepared samples were mounted on a SEM holder by using adhesive carbon tape and carbon conductive paint. Prior to the observations, the specimens were coated with a thin layer of gold (approximately 15 nm) with the use of sputter-coater (Quorum Q150T S). The samples were characterized with the use of the field-emission scanning electron microscope (FE-SEM, Hitachi S-4700).

Transmission electron microscopy (TEM) observations were carried out using a Tecnai Osiris instrument (FEI) with the X-FEG Schottky field emitter operated at an accelerating voltage of 200 kV. Samples for TEM characterization were prepared by the standard procedure [3]. The investigated bacteria strain suspension was washed three times in DPBS, fixed in 3% buffered glutaraldehyde for 24 h. The pellets were washed three times in DPBS, rinsed with 1% osmium tetraoxide solution (DPBS) for 2 h and washed again with DPBS. Samples were dehydrated in the water-alcohol solutions with gradually increasing ethanol concentration (50, 60, 70, 80, 90, 96 and 100%) for 15 min each. The pellets were rinsed with propylene oxide (20 min) and incubated in 1:1 propylene oxide/resin ration overnight (Durcupan, Sigma-Aldrich). Following samples incubation in 100% resin at 37°C for 24 h and then 60°C for 48 h. The samples were sectioned using an ultramicrotome (Leica) equipped with the glass-edged knife (Diatome). The ultrathin lamellas were placed onto Cu TEM slots with the carbon-coated membrane and stained with lead citrate and uranyl citrate for contrast enhancement procedure or UranyLess contrast enhancement solution.

Results and Discussion

The SEM and TEM observations revealed significant differences in cell morphology of observed materials depending on the preparation procedure. The standard fixation for SEM observations (FIG. 1a and b) results in the reduction of cell volume, changes in cell morphology and removal of adsorbed NPs from the cell wall. The protocol for TEM observations, with the UranylLess contrast enhancement results in falsified cell morphology (FIG. 1c) in comparison with the standard procedure (FIG. 1d).

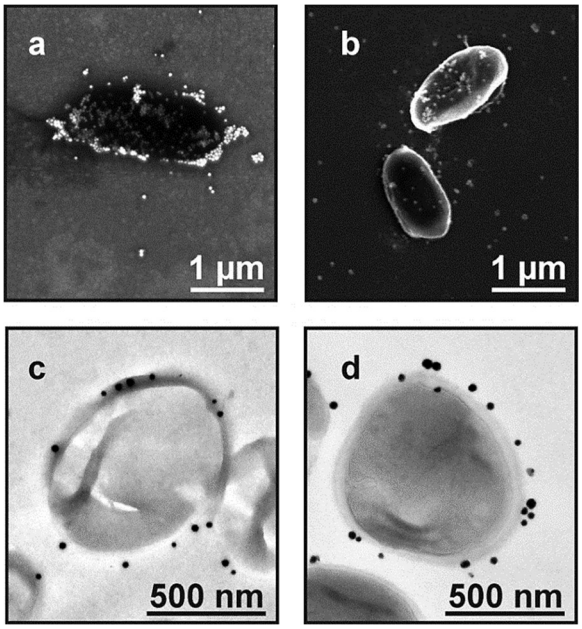

FIG. 1. Electron Microscopy microphotographs of *P. putida* coated with AuNPs. (a) bacteria cultured on the glass, simply dried in air (SEM), (b) bacteria cultured on ITO, fixed and dehydrated (SEM), (c) UranylLess contrast enhancement (TEM), (d) standard contrast enhancement procedure (TEM)

Conclusions

The most effective procedure for SEM observations of bacteria coated with NPs is a protocol, in which glass substrate is used and the probe is simply dried in air, without fixation and dehydration of material. The most appropriate procedure for TEM visualization is the standard one with contrast enhancement using uranyl citrate and lead citrate.

Acknowledgments

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BACTERIA ADHESION TO GRAPHENIC SURFACES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 68]

Introduction

Graphenic materials, due to their specific properties such as excellent electrical conductivity, inherent mechanical strength, thermal conductivity, affinity for heteroatom insertion and biocompatibility are investigated as a materials for many applications including biosensors, drug delivery systems and implants surface engineering, to mentioned a few [1]. The evaluation of the interaction forces between graphene and bacteria, as well as identification of key parameters ruling the adhesion process are of particular importance [2]. Microbial adhesion to surfaces is primarily mediated by non–specific interaction forces. They include van der Waals and electrostatic potentials, which both operate over a long range, as well as hydrophobic and acid-base interactions that act over a shorter range. The mechanism of bacteria attachment to surfaces can be analysed in terms of DLVO (Derjaguin–Landau–Verwey–Overbeek) theory [3]. In this work, we propose the work function measurements by Kelvin probe as a straightforward method for direct evaluation of graphenic surfaces for bacteria colonization. The changes in work function were induced by controlled functionalization of the graphenic surface by oxygen plasma (introduction of surface oxygen groups).

Materials and Methods

The conductive graphene sheets were purchased from Graphene Laboratories, Calverton NY, USA. The oxygen plasma treatment of graphenic samples was carried out using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany). The partial pressure of oxygen, exposure time and the generator power were adjusted to obtain different levels of modification of the electrodonor properties of graphenic surfaces. The contact potential difference (V_{CPD}) measurements were carried out by the Kelvin method with KP6500 (McAllister Technical Services). The modified surfaces were characterized in terms of carbon structure (μRaman spectroscopy), surface morphology (scanning electron microscope), wettability (water contact angle) and surface oxygen concentration (X-ray photoelectron spectroscopy). The changes in bacteria adhesion (*Staphylococcus aureus* 24167 DSM, Gram-positive bacteria) on the graphenic surfaces after oxygen plasma treatment were investigated using a fluorescent microscope (IX51 Olympus). Microbiological tests were performed for three independent series according to the procedure described elsewhere [4]. After observations, 15 randomized fluorescence microscopic images were taken and the area occupied by the bacteria was evaluated using ImageJ 1.50 V software [5].

Results and Discussion

The graphenic surface was modified by oxygen plasma treatment with various parameters (power: 2-60 W, oxygen pressure: 0.14-0.5 mbar, time: 2 s-20 min). The measured Raman spectra of the investigated graphenic materials are typical with the characteristic bands at: ~1580 cm^{-1} (G band) and ~2725 cm^{-1} (2D band). For the samples exposed to plasma for 5 and 20 min, the D band at ~1350 cm^{-1} appears. The XPS results show a substantial increase in oxygen surface concentration from 0.75 at.% (unmodified graphene) up to 6.6 at.% for the longest time of exposure to plasma (t = 20 min, P = 60 W, p = 0.5 mbar). Generation of surface oxygen groups leads to dramatic increase in the work function (from 4.4 eV to 6.0 eV) and decrease in the water contact angle (from 93.8° to 7.0°). In order to check how the surface modification influences the microbiological properties of the graphenic material, the investigated surfaces were evaluated in terms of bacterial colonization. The quantification of the bacteria adhesion to the graphenic surfaces was performed using the surface area occupied by the *S. aureus* after the incubation time of 1 hour (relevant for the initial stage of biomaterial-centered infection after implantation). The bacteria coverage systematically increased with the plasma treatment from 3.2% (untreated graphenic surface) to 9.2% (60 W, 0.5 mbar, 20 min). The trend of bacteria adhesion for the investigated graphenic surfaces follows the tendency observed for the work function modifications (FIG. 1). The obtained results revealed that colonization of graphenic surfaces is strongly governed by the surface oxygen concentration, wettability, and electronic properties (work function).

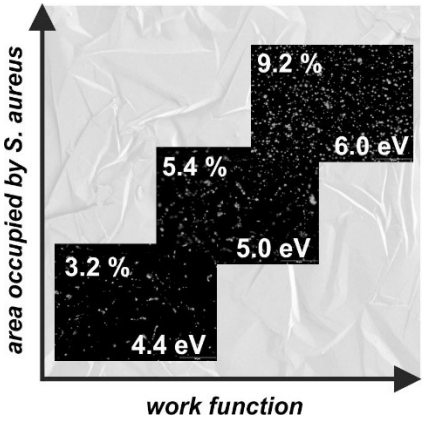

FIG. 1. The general trend observed for *S. aureus* adhesion to graphenic surfaces. At the background the morphology of the graphenic material is shown.

Conclusions

The strong correlation between *S. aureus* adhesion to the graphenic surfaces and electron work function of the prepared samples was discovered. Based on the obtained results the electron work function was proposed as a suitable parameter for the evaluation of graphenic surfaces against bacteria colonization. Introduction of the surface oxygen groups leads to dramatic changes in wettability and work function, so their surface coverage should be controlled, while designing the carbon-based biomaterials.

Acknowledgments

The study was financed by the Polish National Science Centre project awarded by decision number DEC-2016/21/B/ST8/00398. Dr. Michal Mazur is gratefully acknowledged for SEM observations.

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SONOCHEMICAL SYNTHESIS OF DRUG MOLECULES NANOPARTICLES: TOWARDS CONTROLLED DRUG RELEASE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 69]

Introduction

Nanotechnology is rapidly spreading across all fields of science such as electronic, aerospace and medicine. At the nanoscale, physical, chemical, and biological properties differ from the properties of individual atoms and molecules of bulk matter. Therefore, it provides a possibility to develop new, advanced materials which meet the demands of high-tech applications, e.g. in-site controlled drug delivery systems (DDS). Advanced studies of the strategies for drug anchoring and hence, controlled elution kinetics are vividly investigated. For controlled drug delivery systems, numerous approaches are used i.e. micelles, hydrogels, biodegradable polymeric matrix [1]. Sonochemical synthesis is found to be a new, effective method to produce DDS. This technique has been successfully utilized for the synthesis of inorganic nanoparticles [2], while for organic ones, only a few literature reports are available [3-5]. The study aimed to explore one-step sonochemical approach to produce fluorouracil and diclofenac sodium salt nanoparticles and their simultaneous embedding into oxygen plasma modified parylene C surface.

Materials and Methods

Parylene C films were obtained using CVD technique (ParaTech Coating Scandinavia AB). Polymer surfaces were modified using oxygen plasma (FEMTO system, Diener Electronics) to generate oxygen-containing functional groups and nanotopography.
The nanoparticles of fluorouracil and diclofenac sodium salt (Sigma-Aldrich) were formed and subsequently deposited during the one-step process at the surface of plasma-modified parylene C using an ultrasonic generator with the following parameters: frequency 20 kHz, amplitude 30%, and time 6 min. Solutions of drugs in deionized water were 2.5 mg/ml and 15 mg/ml for fluorouracil and diclofenac, respectively. The developed system was thoroughly characterized in terms of particle size (NTA, TEM), surface dispersion (IR-image) and drug release kinetics (UV-Vis).

Results and Discussion

Nanoparticles obtained via sonochemical synthesis were about 100 nm (diclofenac) and 50-100 nm (fluorouracil). In order to get more in-depth insight into the nanoparticles size, TEM observations were conducted. The obtained nanoparticles were amorphous with spherical shape (FIG. 1).
Test of the drugs' stability in ultrasounds was carried out using ATR – IR spectroscopy. There were no significant changes between the spectra of reference samples and those subjected to ultrasound.
The presence of the sonochemically obtained nanoparticles was confirmed using ART – IR technique. The collected spectra revealed characteristic bands at 1644 and 745 cm^{-1} for fluorouracil and diclofenac, respectively. An apparent increase in the absorbance was observed for drug–containing samples when compared to the parent samples of oxygen plasma modified parylene C, indicating the presence and fairly uniform distribution of the NPs. For the average sample (2 cm^2) drug load was estimated to be ~4 μg and 70 μg for fluorouracil and diclofenac, respectively.
The elution studies revealed the strong burst effect of the fluorouracil/parylene C system, where the drug was completely eluted in 30 min. In contrast, the diclofenac/parylene C system provided prolonged drug elution time, reaching seven days.

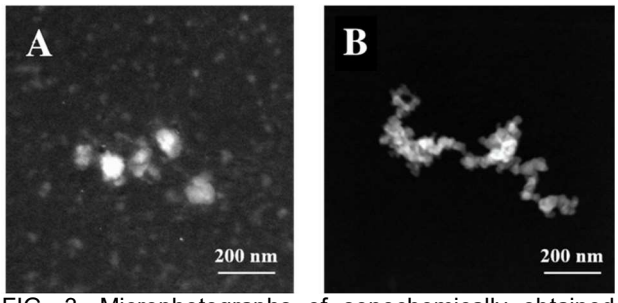

FIG. 3. Microphotographs of sonochemically obtained diclofenac (A) and fluorouracil (B) nanoparticles.

Conclusions

In this study, it was proved that sonochemical production of therapeutic coatings is an alternative, more effective method of producing hybrid systems for controlled in-site drug release. The most important advantages of the sonochemical synthesis are preparation time, increased drug availability for the targeted tissue, lack of chemical waste and toxic solvents.
The presented method is simple, efficient, environmentally friendly and non-destructive in relation to the parylene C surface and drug molecules. The applied sonochemical strategy can be successfully implemented for other drug–polymer coating couples. The sonochemical method is a promising technique in the context of designing novel implantable materials with the controlled drug release function.

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POLY(ACRYLIC ACID) NANOGELS – BASIC REACTIONS AND APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 70]

Introduction

Polyelectrolytes constitute a broad class of polymers including the most important biopolymers as nucleic acids, proteins and many others polysaccharides, as well as synthetic polymers that find numerous applications, e.g. as stimuli-sensitive biomaterials. The object of our research is poly(acrylic acid), PAA is a weak anionic polyelectrolyte of simple chemical structure. Previous studies on PAA radical reactions have focused on identification of radicals and indication of their transformation mechanisms [1,2], overall radical lifetimes [1,3,4] and on the application of intramolecular cross-linking reaction. The aim of this work is to fill important gaps in our understanding of kinetics and mechanism of radical recombination reactions in charged polymers. These gaps, at the kinetic level, include lack of knowledge on the character of the kinetics (classical vs. non-classical order, complexity), on the character of processes determining the reaction rates and on the influence of the reaction conditions on the reaction kinetics. Radicals on PAA chains are generated, by applying a short pulse of ionizing radiation in the form of high-energy electrons from an accelerator, to the deoxygenated polymer solution. Their fate can be then followed by a selected real-time fast spectrophotometric detection technique without any interference from other effects. No initiators are needed. One of the reactions during irradiation of acidic PAA solutions is intramolecular cross-linking, which leads to internally cross-linked polymer nanoparticles, i.e. nanogels [2,4-6], which are intensely studied for applications in nanomedicine. The second aim was to couple PAA nanogels with peptide molecules – bombesin with additional chelating groups, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, DOTA to obtain a radioisotope nanocarrier having selective affinity for certain types of tumor cells. In the future such carriers could be used as radiopharmaceuticals.

Materials and Methods

The first step was radiation-induced synthesis of nanogels and characterization their physicochemical properties. Dilute aqueous solutions of poly(acrylic acid) from 10 mmol dm^{-3} to 25 mmol dm^{-3} were prepared by stirring at 50°C for 12h. The pH of PAA solutions before irradiation was set to 2.0 ($HClO_4$). Solution was irradiated by short pulses of fast electrons with pulse duration 2 μs, pulse frequency 0.5 Hz, electron energy 6 MeV, absorbed dose of ionizing radiation per single pulse: 1 kGy, generated by an ELU-6 linear accelerator (Eksma, Russia). The solutions during irradiation were circulating at 1 cm^3 s^{-1} in a gas-tight (Ar atmosphere), closed-loop system, passing through a quartz cell.

The physicochemical properties as size and weight-average molecular weight were determined by static multiangle laser light scattering measurements and dynamic light scattering measurements (25.0+0.1 °C, in aqueous solution of 0.5 mol dm^{-3} $NaClO_4$, pH 10).

The second step was nanogel functionalization with peptide molecules. PAA nanogels with suitable parameters were coupled with bombesin (with additional chelating groups) having selective affinity for certain types of tumor cells. The physicochemical properties of nanogels coupled with bombesin-DOTA were analysed by UV-VIS, FT-IR and ^1H NMR spectroscopy.

In parallel to this work, pulse radiolysis experiments, employing spectrophotometric detection were performed to estimate the lifetimes of radicals under the variety of conditions (pulse duration 0.5 μs). Kinetic studies were performed by classical pulse radiolysis [7].

Results and Discussion

During irradiation of dilute aqueous polymer solutions, ionizing radiation is predominantly absorbed by water and the result is water radiolysis, leading to hydroxyl radicals and hydrogen atoms, capable of abstracting hydrogen atoms from polymer chains in a very fast, diffusion-controlled reaction, which leads to the formation of polymer radicals. Preliminary studies indicate a very significant effect of pH on the lifetime of PAA radicals and that if we irradiate this polymer in solution so that on average more than one radical is formed on the chain, the kinetics of radical decay shows significant deviations from the classical second-order kinetics. Moreover, PAA nanogels synthesis is possible at low pH, when carboxylic groups are protonated; at higher pH Coulombic forces between PAA chains are very strong and chains scission dominates over cross-linking [1]. Measurements have confirmed that by adjusting PAA concentration and absorbed dose, one can control the molecular weight and dimensions of nanogels. PAA nanogels of suitable parameters were functionalized by coupling with bombesin-DOTA compound using a coupling agent. The research confirmed that nanogels were coupled to bombesin-DOTA.

Conclusions

Radiation synthesis of nanogels allows to obtain nanocarriers for radiopharmaceuticals without potentially harmful chemical substances, like initiators or catalysts. The only substrates are water and polymer. The obtained results on functionalization of the nanogels indicate that peptide molecule was efficiently coupled to the nanogels.

Work is under way on: (1) kinetic and mechanistic studies to provide better understanding of free-radical chemistry of the studied system and (2) radiolabeling of the radiation-synthesized PAA nanogels, in order to obtain selective nanocarriers for use in radiation therapy.

Acknowledgments

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GMP COMPLIANT ISOLATION AND CULTURE OF HUMAN ADIPOSE TISSUE- DERIVED MESENCHYMAL STEM CELLS FOR APPLICATIONS IN TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 71]

Introduction

Tissue engineering refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. For clinical applications, all the component must be manufactured in accordance with Good Manufacturing Practice (GMP) [1]. Subcutaneous human adipose tissue (AT) is an abundant source of mesenchymal stem/ stromal cells (MSCs). In comparison to bone marrow-derived MSCs, AT-MSCs occur at a 100–1000-fold higher frequency within adipose tissue on a volume basis [2]. MSCs can differentiate into multiple tissues including bone, cartilage, muscle etc. Moreover, it has been proposed that the functional benefits observed after MSCs transplantation in experimental models of tissue injury might be also related to the secretion of soluble factors acting in a paracrine fashion *e.g.* exhibiting immunosuppressive properties [3]. Thus, the goal of this study was to optimize isolation and culture methods of MSCs derived from adipose tissue in accordance with GMP rules for further applications in tissue engineering.

Materials and methods

Adipose tissue were obtained during liposuction from adult young male and female donors. To get rid of erythrocytes and other decontaminations, adipose tissue were washed several times in PBS (w/o Ca^{2+}, Mg^{2+}) supplemented with antibiotics and antimycotic. Then, after enzymatic digestion using GMP- grade collagenase stromal vascular fraction (SVF) were isolated and seeded onto cell culture flasks in three commercially available GMP-grade cell culture media (serum- free, animal component free). After removal of non-adherent cells, adherent fraction (AT-MSCs) were expanded until 5 passage. Morphology of adherent cells were monitored by phase- contrast microscopy. Identification of these cells were carry out in accordance with the International Society for Cellular Therapy (ISCT) position statement paper on antigenic profile and multipotency differentiation potential [3]. During optimization process, different concentrations of enzyme, culture media as well as culture conditions were examined. To evaluate potential application of AT-MSCs in regeneration of damaged cartilage, cells were differentiated into chondrocyte under high pressure (2 PSI, 5 PSI) and low oxygen (5%) conditions using Avatar Cell Control System. In *in vivo* study, selected dose of AT-MSCs were suspended into hyaluronic acid and injected to the injured pigs' knee to evaluate regenerative potential of these cells.

Results and Discussion

SVF with viability greater than 95% were obtained after isolation step. The yield of isolation were high and comparable between isolations (2,1 x 10^5 cells/ 1g of lipoaspirate). We selected the optimal enzyme concentration as well as the best culture medium. The kinetics of growth and proliferation of AT-MSCs were also comparable between isolations. The isolated cells: 1) were plastic- adherent when maintained in standard culture conditions; 2) possess phenotype characteristic for MSC cells ($CD45^-$, $CD34^-$, $CD14^-$, $CD11b^-$, $CD19^-$, HLA-DR-, $CD105^+$, $CD44^+$, $CD73^+$, $CD90^+$); 3) exhibit ability to differentiate into adipocytes, chondrocytes and osteoblasts; confirming their identity according to ISCT recommendations. Moreover, AT-MSCs can effectively differentiate into chondrocytes when culture under high pressure and low oxygen conditions mimicking the condition observed in the knee. AT-MSCs suspended into transport medium improving their viability, can be then suspended into fluid carrier such as hyaluronic acid and use in tissue engineering.

Conclusions

We successfully optimized efficient protocol for isolation and culture of AT-MSCs using GMP- grade reagents and materials as well as define interoperation control and release criteria. AT-MSCs due to their ability to differentiate into chondrocytes and secretion of wide range of bioactive factors can be effectively use in tissue engineering.

Acknowledgments

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INVESTIGATION ON THE INFLUENCE OF ZrC COATINGS STRUCTURE ON THEIR RESISTANCE TO CORROSION AND ANTIMICROBIAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 72]

Introduction

Coatings deposited on the surface of metallic biomaterials used in orthodontics and jaw implantology should meet special requirements, i.e., in addition to high biocompatibility, should be characterized by good corrosion resistance in mouth environment, good adhesion to the substrate and resistance to brittle fracture, as well as be resistant to microbial growth. Particularly noteworthy in these applications are nanocomposite coatings of the nc-MeC/a-C structure, where Me = Ti, Zr, W, Nb etc. deposited by vacuum-plasma techniques, especially PVD (Physical Vapor Deposition), because they can exhibit particularly advantageous mechanical properties (high hardness, low wear ratio due to friction and good adhesion state to the substrate). These properties depend on the phase composition and structure of the coatings, which are a function of the carbon concentration in the coating and parameters of the deposition process [1,2]. The object of research presented in this article are thin coatings based on ZrC with different concentration of carbon, deposited on substrates made of 304L medical steel. The aim of the research is to determine the optimal composition of the coating due to its mechanical, anti-corrosion and antimicrobial properties in terms of its use for covering biomaterials used in orthodontics and jaw implantology.

Materials and Methods

ZrC coatings were deposited on 304L steel substrates by reactive magnetron sputtering at 400°C and -10 V substrate bias voltage. The coatings were deposited from the metallic Zr target in Ar + C_2H_2 atmosphere at C_2H_2 flow rate of 3.5; 4.5; and 6.5 sccm, thus obtaining coatings with different total carbon concentrations. The evaluation of the corrosion resistance of the systems was carried out using potentiodynamic polarisation tests using the device ATLAS 0531 Electrochemical Unit, in a system of a conventional electrochemical three-electrode cell, where working electrode was a sample with the active area of 0.292 cm^2, reference electrode – saturated calomel electrode (SCE, $Hg/Hg_2Cl_2/KCl$) and counter electrode – platinum sheet. The corrosive medium (electrolyte) was Fusayama-Meyer artificial salvia. The tests were carried out at the temperature 25±1°C with the scan rate of 0.001 V/s. Measurements were repeated until obtaining three similar results – polarisation curves. Corrosion potential (E_{corr}), corrosion current density (i_{corr}) and polarisation resistance (R_{pol} – a value inversely proportional to i_{corr}) have been estimated by the Tafel extrapolation method [3]. The static contact angles were measured through the sessile drop method using F4 Gomeiometer by Rame-Hart Instrumental Co. Drops with a volume of approximately 4 µl were deposited onto the investigated surfaces using a microsyringe. Fusayama-Meyer's artificial saliva, was used as a test liquid. The contact angles were measured at 3–5 different points at the surface of each sample. Seven bacterial species representative of the peri-implant and oral cavity environment were used in studies of antimicrobial properties: *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. The assessment of antibacterial activity was made using the direct method based on the criteria contained in SN 195920 standard, while the evaluation of antifungal activity was performed based on SN 195921 standard. The susceptibility of the surface to microbial adhesion was assessed using fluorescent staining and observation in a Motic BA410 E fluorescence microscope [4,5]. The assessment of the adhesion of coatings to the substrate and their resistance to cracking was carried out using the Rockwell C test.

Results and Discussion

On the basis of polarization curves, current values and polarization resistance were estimated. The most favorable values of these parameters, from the point of view of corrosion resistance (the highest potential and polarization resistance and the lowest current density among the tested) were observed for the coating with the highest total carbon concentration. Also, based on microbiological tests, it was found that the best bacteriostatic effect and the highest resistance to colonization with microorganisms, characterized ZrC coatings with a higher carbon concentration of 61 and 75% at, obtained at the C_2H_2 flow rate, 4.5 and 6.5 sccm respectively. In turn, on the basis of carried out indentation tests, it was found that as the concentration of carbon in the coating increases, resistance to cracking decreases and the adhesion of the coating to the substrate deteriorates. Additionally, based on the measurements of the contact angle, it was found that the surfaces of all substrate/coating systems exhibited poorer wettability than the steel substrate.

Conclusions

Based on the conducted tests, it was found that all substrate/nc-ZrC/a-C coating systems have significantly better corrosion resistance in the artificial saliva solution than 304L steel substrate (they show several dozen times lower corrosion current and higher polarization resistance in potentiodynamic tests). In the case of the coating with the highest carbon content, in the potentiodynamic studies, no breakdown potential was observed, which may indicate a high resistance of the tested system to pitting corrosion. Coatings with a total carbon concentration above 60% are static for pathogenic microorganisms and have low colonization potential, compared to 304 L medical steel. In summary, the tested ZrC coatings are promising candidates for metal implant coverings, and their use in orthodontics requires the selection of optimal carbon concentration due to the mechanical, antimicrobial and anti-corrosion properties of the substrate/coating system.

Acknowledgments

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INVESTIGATION OF MECHANICAL AND ANTICORROSIVE PROPERTIES OF ZrC COATINGS DEPOSITED BY MAGNETRON SPUTTERING TECHNIQUE

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[Engineering of Biomaterials 148 (2018) 73]

Introduction

One of the key aspects in the field of biomaterials production is the modification of their surface, among others by using vacuum-plasma techniques. In particular, on a large scale are successfully used the PVD (Physical Vapor Deposition) methods for the deposition of protective coatings of various structures and compositions. On the surface of biomaterials using these methods, coatings based on TiN, TaN, ZrN, TiC, TaC, TiO_2, TaO, TiCN, a-C, ZrCN or ZrCN-Ag are deposited [1-4]. Among them, more and more researchers' attention is focused on coatings based on ZrC and ZrCN. The aim is to optimize the properties of these coatings by selecting the appropriate version of deposition method (among others radio-frequency (RF) magnetron sputtering (MS), pulsed laser deposition (PLD), etc.) and optimization of process parameters for the selected version. Taking into account the potential use of ZrC based coatings, inter alia, in orthodontics, it becomes very important to know the anti-wear and anti-corrosion properties of these coatings deposited on medical steel. Therefore, the analysis of the effect of carbon concentration in ZrC coatings on these properties was adopted as the main goal of the research.

Materials and Methods

The object of research were ZrC coatings deposited on 304L steel substrates by reactive magnetron sputtering at 400°C and -10 V substrate bias voltage. The coatings were deposited from the metallic Zr target in Ar + C_2H_2 atmosphere at C_2H_2 flow rate of 2.5, 3.5; 4.5; and 6.5 sccm, thus obtaining coatings with different total carbon concentrations. In order to determine the mechanical properties of the coatings, a number of indentation tests were performed using the Berkovich and Rockwell indenters. The tribological properties of coatings were assessed basing on the so-called ball on disk test. On the basis of cross profiles of the wear track, volumetric wear ratio and friction coefficients of the coating with alumina ball were determined. In addition, a detailed analysis of the state of internal stresses in the ZrC/steel substrate and ZrC/Si substrate/coating systems was made using X-ray diffraction and a method based on measuring the radius of curvature of the sample with the deposited coating. Parallel, the analysis of anti-corrosion properties of the tested coatings, in the artificial saliva solution, using potentiodynamic polarisation tests was carried out. The results of corrosion tests were confronted with the results of mechanical and tribological tests.

Results and Discussion

Basing on the indentation curves (load vs. penetration) and numerical simulations based on FEM (finite element method), one were determined for coatings with different carbon concentrations, among others: Young's modulus, yield point and tangent modulus. In particular, it was shown that for the carbon concentration in the coating C=51 at. % (C_2H_2 flow rate approx. 2.5 sccm) the hardness and Young's modulus of the tested coatings reach maximum values (respectively H=40 GPa, E=350 GPa), and in case of further carbon concentration increase both values of hardness and Young's modulus decrease. Obtained results of indentation tests were also used in the analysis of the state of adhesion of the coating to the substrate and the assessment of fracture toughness. It was found that the coatings with the highest carbon concentration are characterized by the worst adhesion to the substrate from the tested coatings and posses the low resistance to cracking. Analyzing the results of internal stresses in the coating/substrate system obtained with XRD and based on the measurement of the radius of curvature of the sample with deposited coating, it was found that there was a maximum compressive stresses (about 4.5 GPa) for coatings with a carbon concentration C=56.5 at. % (C_2H_2 flow rate approx. 3.5 sccm). Increase in carbon concentration to C=84 at.% (C_2H_2 flow rate approx. 6.5 sccm) caused a nearly two times decrease in the value of compressive stresses (about 2.3 GPa).

Conclusions

On the basis of the conducted research, the evolution of phase composition of the ZrC coating, deposited on the medical steel by the magnetron sputtering method in the C_2H_2 atmosphere, along with the increase of the carbon concentration was determined. In particular, it has been shown that with increasing carbon concentration the proportion of amorphous carbon surrounding the nanocrystalline ZrC carbides increases. This change in the phase composition of the coating causes a decrease in the coefficient of friction and a decrease in the wear coefficient of the coating in the ball on disk test. Parallel, along with the increase in carbon concentration, a decrease in fracture toughness and deterioration of the adhesion of the coating to the substrate was observed. Independently carried out corrosion tests of the analyzed systems: 304L steel/ZrC coating, confronted with mechanical and tribological tests, allowed to determine the phase structure and coating structure with optimal properties from the point of view of metal implants, including orthodontic wires.

Acknowledgments

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THE PROPERTIES OF NANOSILVER – DOPED NANOHYDROXYAPATITE COATING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 74]

Introduction

The titanium alloy (Ti6Al4V, Ti6Al7Nb and Ti13Zr13Nb) nowadays are the most frequently used materials for orthopedic implants because of their high biocompatibility, high corrosion resistance in body fluids environment and proper mechanical properties [1].
To improve biocompatibility and mechanical properties, the surface modification techniques like laser treatment, hydroxyapatite or nanohydroxyapatite coatings deposition or nanooxidation were used. Electrophoretic deposition technique could be applied to obtain thin films of hydroxyapatite and nanohydroxyapatite. To provide antibacterial properties of the hydroxyapatite coatings, the doping of nanosilver or nanocopper nanoparticles with similar bactericidal effects as antibiotics was investigated [2,3].
The aim of this research was to study the properties of nanohydroxyapatite (nanoHAp) and nanohydroxyapatite doped with nanosilver (nanoHAp/nanoAg) coatings obtained by an electrophoretic deposition process on the Ti13Zr13Nb.

Materials and Methods

The specimens made of the Ti13Zr13Nb alloy were polished with abrasive paper, grid 2000# as the last.
The electrophoretic deposition (EPD) was carried out in a suspension prepared by dispersing 0.1 g of HAp nanopowder with average particle size about 20 nm (MK Nano Canada) (specimen “a”) and 0.1 g of HAp nanopowder and 0.01 g of nanosilver with average particle size about 30 nm (Hongwu International Group LTD, China) (specimen “b”) in 100 mL of anhydrous ethanol. The suspensions were obtained by ultrasonic mixing for 60 min at room temperature. The electrodes were placed parallel to each other within a distance 10 mm and connected to the DC power source (MCP/SPN110-01C, Shanghai MCP Corp., China). The electrophoretic deposition was performed at 30 V for 2 min at room temperature. Specimen were air dried at room temperature for 24 h. Finally, the coated Ti13Zr13Nb specimens were thermally treated in a tubular furnace (PROTHERM PC442, Ankara, Turkey) in a vacuum at 800°C for 120 min.
The coatings` surfaces and cross-sections were observed using a high resolution SEM (JEOL JSM-6480).

Results and Discussion

FIG. 1. shows the SEM images of nanoHAp and nanoHAp/nanoAg coatings. The agglomerations of nanoHAp powder and cracks appear on both types of coatings, in particular on the nanoHAp coating, for which a greater number of longer cracks is seen.
FIG. 2. illustrates the thickness of the coatings. The thickness of the nanoHAp coatings was 3.84 ± 0.43 μm and of nanoHAp/nanoAg was 2.29 ± 0.32 μm.

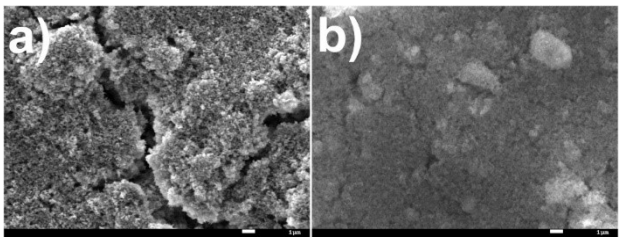

FIG. 1. SEM images of the nanoHAp (a) and nanoHAp/nanoAg (b) coatings after thermal treatment.

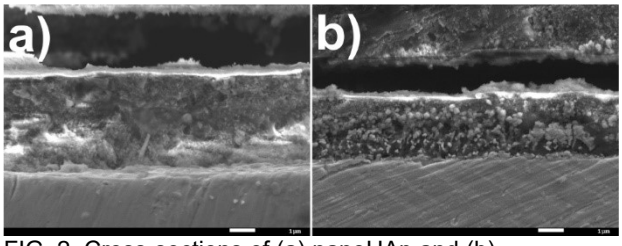

FIG. 2. Cross-sections of (a) nanoHAp and (b) nanoHAp/nanoAg coatings after thermal treatment.

The agglomeration of nanoHAp particles probably is due to faster kinetics of the migration process and low time to find and occupy the most suitable positions to form a uniform coating [4,5]. NanoHAp/nanoAg coating compared with the nanoHAp coating is characterized by a smaller number of cracks what may be due to the presence of silver nanoparticles inside the coating and reducing the internal stress inside the coating after the thermal treatment. The cross-sections illustrate that both nanoHAp coatings are well adjacent to the titanium substrate without any delamination and that nanosilver presence reduces thickness of coating. This effect is probably caused by disturbed migration of less conductive nanoHAp powder particles by perfect conductive nanosilver particles.

Conclusions

The electrophoretic deposition process (EPD) is the method which allows for obtaining homogeneous, thin nanoHAp/nanoAg coatings on the Ti13Zr13Nb alloy. The presence of nanosilver particles has a significant effect on homogeneity, quality and thickness of the nanohydroxyapatite coatings.

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NEW APPROACHES IN THE ORTOBIOLOGICAL THERAPY

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[ENGINEERING OF BIOMATERIALS 148 (2018) 75]

Abstract

Osteoarthritis is the most frequently reported and the most onerous disease in middle-aged and elderly people. Degenerative changes constitute a heterogeneous set of pathological transformations of the structure and function of the elements forming the joint, which is the result of factors disturbing their balance. The loss of joint cartilage mass with its insufficient rebuilding or transformation of subchondral bone and other soft tissue structures is characteristic for this disease [1-3].

Nowadays, in addition to the development of invasive surgical methods aimed at cartilage reconstruction, it is possible to use ortobiological therapy in parallel, taking into account the careful qualification for this type of treatment. Ortobiological therapies use the vital potential of the unique tissue that is blood. The expected effects of ortobiological treatment in osteoarthritis is joint cartilage regeneration and acceleration of healing [1,2,4].

The effectiveness of each therapy depends on a number of factors that affect its results not only during or after its application, but also before the start of treatment. Therefore, it is important to properly prepare the tissues for the planned therapy. Targeted physical activity, taking into account the correctness of its performance is extremely important in the preparation of the tissue base for the application of autologous preparations. Physical exercises, preceding the application of injection within the musculoskeletal system, improve the effectiveness of ortobiological treatment. The combination of ortobiological therapy and subsequent physiotherapy support the rehabilitation process, leading to the achievement of the best possible end results [5-7].

The platelet rich plasma (PRP) is a more and more commonly used method of ortobiological therapy. Platelet rich plasma is a source of growth factors that is used to improve the function of tissues by remodeling their morphology and improving metabolism. This therapy distinguishes itself from conventional means of influencing tissue and contributes to establishing a new therapeutic direction in the treatment of osteoarthritis [8].

Another therapy used in ortobiology is autologous conditioned serum (Orthokine®). This modern method of treating inflammation and degenerative joints strengthen the integrity of joint cartilage by introducing anti-inflammatory cytokines into the body. In addition, a significant increase in the concentration of growth factors during conditioning using Orthokine® has been demonstrated. These factors stimulate tissue repair and regeneration processes by migrating cells to the site of damage, their multiplication, differentiation and the formation of new blood vessels [9-11].

One of the methods used to treat osteoarthritis is stem cell therapy. The purpose of these cells is to replace the dead cells and rebuild the tissue. Due to the fact that stem cells present in the human body, as the body ages, they lose their ability to intensively regenerate, the method of stem cell proliferation is used for therapeutic purposes. Properly prepared stem cells are injected precisely to the affected area. Stem cell therapy is a method that is constantly improved both in the selection of the source of cells and the method of their preparation in order to isolate and concentrate the cells. The research carried out in this direction is aimed at optimizing the therapy and, consequently, effective assistance to patients with osteoarthritis [12].

An important problem associated with osteoarthritis is the occurrence of degenerative cysts. They appear due to the formation of bloody strokes and the conduct of destructive processes in the subchondral bone. Ongoing work on surgical methods to prevent the rapid progression of the disease. Currently, tissue scaffolds are used to fill defects. These are the resorbable material that fills the tissue defect, and through their structure they allow for the migration of cells and overgrowth of tissues, and thus its reconstruction [13-15].

In order to improve the properties of materials, research is conducted on hybrid implants that would combine individual therapies. The possibility of comprehensive treatment, consisting in filling cavities with an implant, with the use of an ortobiological preparation in the scaffolding space, will allow bone tissue remodeling to proceed under optimal conditions [16].

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MicroRNAs IN ATHEROSCLEROSIS: ALTERED EXPRESSION AND DIAGNOSTIC POTENTIAL

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Introduction
MicroRNAs are a group of small, non-coding RNAs, occurring in all species. MicroRNAs are very important regulators of protein coding genes expression. Incorrect regulation of gene expression by microRNA is the cause of many diseases.

Materials and Methods
Peripheral blood mononuclear cells were obtained from patients with atherosclerosis. miRNA levels were determined using next generation sequencing, NGS (S5 XL ThermoFisher Scientific technique). Statistical analyses identified differences between normal and patients samples. miRNA expression profiles that associate with atherosclerosis were established. A variable was considered statistically significant if $p < 0.05$. All statistical calculations were performed using the R software.

Results and Discussion
Differential expression analysis revealed around 30 microRNAs with highly significant changed expression in patients ($P < 0.0001$), in comparison to healthy individuals. Around 20 out of those microRNAs were showed to be related to atherosclerosis for the first time and were embodied in patent application No. P. 424674. Moreover, around 40 high confident gene targets were determined for found microRNAs using predictive target annotation databases and confirmed in transcriptomic analysis of study participants.

Conclusions
We have found significantly differentially expressed miRNAs in atherosclerosis. Recognition of dysregulations in microRNA regulatory network, associated with atherosclerosis development, allows to discover new mechanisms involved in etiology of this disease and enables to establish novel biomarkers, providing new diagnostic and therapeutic opportunities.

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ENGINEERING OF DEXTRAN-BASED MATRICES FOR SOFT TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 77]

Introduction

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

Chemical structure of dextran enables wide range of chemical modification. Incorporation of crosslinkable moieties (e.g. methacrylic groups, -MA) into dextran structure allows to obtain derivatives capable for crosslinking initiated by UV or ionizing radiation [4-6].

The radiation technique is very efficient and clean tool for modifying polymers. Unquestionable advantages of using radiation include possibility of processing materials in any physical state, at a convenient temperature (usually room temperature), typically with no need of application of additional chemicals, i.e. potentially toxic initiators or catalysts [7]. Moreover, if sufficient dose is applied (typically 25 kGy) sterilization can be accomplished simultaneously with the processing. In our recent study we have demonstrated possibility of radiation synthesis of hydrogels based on biocompatible dextran methacrylate (Dex-MA) [6].

The current work was aimed at synthesizing a dextran derivative having substituents capable of covalent crosslinking (Dex-MA – dextran methacrylate), and to develop conditions suitable for formation of macroscopic hydrogels using radiation technique.

Materials and Methods

Dextran derivatives have been synthesized using the procedure of van Dijk-Wolthuis by coupling glycidyl methacrylate with this polysaccharide, yielding Dex-MA of various degrees of methacrylate substitution (DS) [8]. Dextran (from *Leuconostoc ssp*., Mr = 25,000; 70,000 and 500,000) was purchased from Sigma-Aldrich (Canada), dimethyl sulfoxide (DMSO, 99.5%) and hydrochloric acid (HCl, 36-38%) were obtained from Chempur (Poland). Glycidyl methacrylate (GMA 97%, stabilized by 0.005% hydroquinone monomethylether) was purchased from Sigma Aldrich, 4-(*N,N*-dimethylamino)pyridine (DMAP) were obtained from Sigma Aldrich (USA). Synthesized dextran derivatives were characterized using FTIR and NMR spectroscopy. Dextran-based hydrogels were manufactured in aqueous solutions of Dex-MA through polymerization/crosslinking of methacrylic groups with radiation initiation. Aqueous solutions of 1, 2, 3 and 5% Dex-MA with different DS were prepared, saturated with argon and subsequently irradiated by electron beam (0.5 – 25 kGy). Following the irradiation, the samples of permanent chemical hydrogels underwent sol-gel analysis to determine equilibrium degree of swelling in deionized water (EDS) and gel fraction (GF). Moreover, for selected samples (3%, 25 kGy) cytotoxicity evaluation based on XTT test was performed. The targeted cells used were mouse fibroblasts L929 (European Collection of Authenticated Cell Cultures (ECACC)).

Results and Discussion

The main goal was detailed study on radiation-initiated synthesis of dextran-based hydrogels. Crosslinking of Dex-MA in aqueous solutions was found to be an efficient process yielding gels with high insoluble fraction content (up to 100 %). The equilibrium swelling encompasses a wide range of 20 – 120 g of water absorbed per g of dry crosslinked polymer. Based on collected data it can be concluded that the utility characteristics of hydrogels can be tailored by appropriate selection of parameters such as dextran's initial molecular weight, DS, concentration and irradiation conditions. Moreover, XTT tests have shown that cell viability maintained the level of positive controls for gels of Dex-MA of low DS, 0.15. Hydrogels manufactured from Dex-MA of the lower DS under applied experimental conditions have no negative impact on cell proliferation and viability, therefore can be regarded as non-cytotoxic.

Conclusions

In this work a series of Dex-MA was synthesized by reaction of the polysaccharide with GMA. Irradiation of aqueous solution of Dex-MA in absence of low-molecular-weight additives (crosslinkers) resulted in formation of permanent macroscopic hydrogels even at doses as low as 0.5 kGy. Thus, obtaining hydrogel of this natural polymer using ionizing radiation, i.e. crosslinking through unsaturated C=C bonds of -MA substituents, seems to be interesting alternative in comparison to other methods (chemical and UV-crosslinking). End-characteristics of hydrogels can be tailored by manipulation of crosslinking conditions. Moreover, lack of toxicity of synthesized hydrogels was proved. This, combined with well-known biological activity and functionality of dextran, implies possibility of biomedical applications of these dextran-based hydrogels, especially in the field of soft tissue regenerative medicine.

Acknowledgments

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POLYMERIC HYDROGELS FOR CARTILAGE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 78]

Introduction

Trauma, arthritis and sports-related injuries can damage cartilage and subchondral bone tissues. It has been reported in literature that almost 60% of patients examined by knee arthroscopy show cartilage damage, and ca. 15% of people over 60 years of age have some clinical symptoms of cartilage damage [1,2]. Cartilage tissue has limited capacity for intrinsic repair due to lack of vascularization, innervation, lymphatic networks, and progenitor cells [3]. Current strategies to cartilage tissue defects have evolved from surgery (e.g. microfracture), through osteochondral transplantation, and recently to cell-based repair techniques [4]. To enhance the reconstruction process of the damaged cartilage and bone tissues, it is important to obtain scaffolds able to mimic the features of the tissues and to transport efficiently cells and growth factors. Ideal scaffolds of cartilage tissue should be porous, biocompatible, and capable of promoting cell differentiation and new tissue formation. Very important are mechanical properties that should be stable during degradation of scaffold in response to the formation of new tissue [3]. Hydrogels are promising materials for the delivery of cells and bioactive molecules due to their viscoelastic properties and water-swollen structure [5]. Sodium alginate (SA), a well-known natural polysaccharide composed of α-l-guluronate (G) and β-d-mannuronate (M) residues, is widely used in different biomedical applications, e.g. as scaffolds that can deliver cells and bioactive molecules. One of the advantages is the possibility of hydrogels formation with divalent cations such as calcium or magnesium ions [6]. In our work alginate hydrogels reinforced with graphene oxide (GO) or/and hydroxyapatite (HAp) were investigated as potential biomaterials for cartilage tissue regeneration.

Materials and Methods

For preparation of hydrogels, sodium alginate from *Acros ORGANICS*, calcium chloride from *POCh Avantor*, graphene oxide (GO) in the form of paste from ITME and hydroxyapatite (HAp) from *mkNano* have been used.
Three series of samples (with GO, HAp and both GO and HAp) have been prepared using 3% aqueous solution of sodium alginate cured with 0,075M solution of calcium chloride. GO of concentration was 0.1-3% and 1-30% of HAp. Modifiers were added to sodium alginate solution before curing. The ball shape samples were left for a week in $CaCl_2$ solution in order to obtain fully crosslinked hydrogels.
The obtained hydrogels were investigated using spectroscopic, microscopic and thermal analysis methods. Moreover, investigations of mechanical properties, wettability, tribology, *in vitro* chemical stability *(in PBS and Ringer solution)* and preliminary assessment of bioactivity were performed.

Results and Discussion

SEM observation showed no agglomeration of GO, whereby different result was obtained in case of HAp - this modifier was easily seen on the surface of alginate hydrogel. Noteworthy, it can be helpful in case of reaction with the bone situated underneath the articular cartilage.
The FTIR data confirmed that an addition of GO and HAp influence the alginate matrix; both GO and HAp nanoparticles weakened hydrogen bonds which can be the result of increasing the distance between alginate macrochains. TG and DSC analyses results showed that even a small amount of modifier changes the thermal properties of hydrogels - FIG. 1.

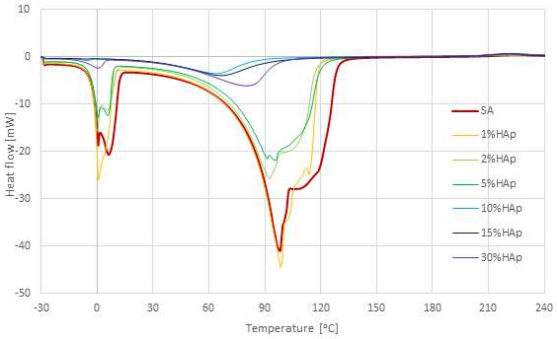

FIG. 1. DSC curves of alginate hydrogels modified with HAp.

TG analysis showed low tolerance to temperature and confirmed high water contents in hydrogels. The wettability test made on alginate films with various alginate content revealed high hydrophilicity of the material as the drop situated on the surface spilled immediately. The contact angle was estimated at around 10°. Moreover, it was observed that the applied modifications strongly influence mechanical properties of alginate hydrogels.

Conclusions

Based on the obtained results it can be concluded that addition of GO or/and HAp considerably change the properties of alginate hydrogels. The obtained hydrogels can be considered as promising materials in modern cartilage tissue engineering.

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HYDROGELS BASED ON NATURAL POLYMERS FOR CARTILAGE TISSUE REGENERATION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 79]

Introduction

Cartilage tissue is composed of chondrocytes and protein fibers. It is a delicate tissue susceptible to deformation, and it has rather little possibility of regeneration. Due to insufficient methods of treatment of damaged cartilage tissue, new solutions are being sought for this problem including application of polymeric hydrogels. In our work we present hydrogels based on sodium alginate modified with both graphene oxide and hydroxyapatite for cartilage tissue regeneration. Sodium alginate shows low toxicity and biocompatibility. Moreover, its properties can be modified through crosslinking and/or incorporation of additives. HAp was added to the alginate matrix, due to its good biocompatibility and ability to stimulate bone regeneration in small bone defects. Another used additive was graphene oxide, which is characterized by exceptional mechanical properties and a large specific surface area. Graphene oxide presence has also a positive effect on cell adhesion.

Materials and Methods

Hydrogels have been obtained using sodium alginate (Acros ORGANICS), anhydrous calcium chloride (POCH), distilled water, graphene oxide (GO) in the form of a paste (ITME) and hydroxyapatite (HAp) from MKNano. The concentration of GO and HAp in hydrogels is presented in Tab. 1.

Sample	1	2	3	4	5	6	7
HAp [%]	30.0	15.0	10.0	5.0	2.0	1.0	0
GO [%]	0	0.1	0.2	0.5	1.0	1.5	3.0

The obtained hydrogels were subjected to a compressive strength test. FTIR and DSC analyses were performed, as well as the preliminary bioactivity and in vitro chemical stability were also investigated.

Results and Discussion

Mechanical tests of unmodified alginate hydrogels show that the best mechanical properties exhibited hydrogel obtained from 3% water solution of sodium alginate crosslinked with 0.075M $CaCl_2$ solution.

The FTIR analysis showed a shift of bands towards higher wave numbers for the C=O group. This may indicate that the polymer chains become looser and the hydrogen bonds get weaker.

DSC studies showed water melting in the hydrogel samples and next water evaporation. The highest heat of melting was observed for the sample containing 0% HAp + 3% GO, the smallest for 2% HAp + 1% GO material. The greatest heat of evaporation was found for hydrogel containing 2% HAp + 1% GO and the smallest 30% HAp + 0% GO.

The bioactivity study showed that only samples containing 5% HAp + 0.5GO and 10% HAp + 0.2% GO were observed to have small amounts of apatite under the chloride layer – FIG. 1. For the chemical stability study, changes in pH were recorded during the incubation of samples in PBS. The mass of samples in PBS fluid increased by approximately 30% after 4 days and remained at this level until the end of incubation. In case of incubation in Ringer's fluid, the pH of the samples increased slightly. Similarly to the incubation in PBS, the mass of samples increased by about 35%.

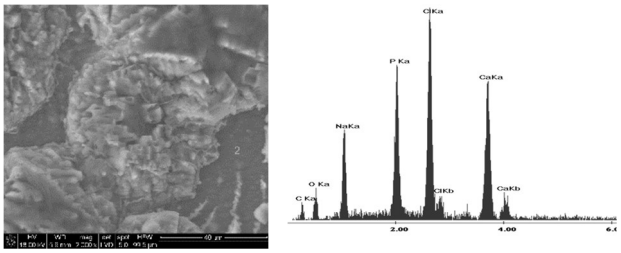

FIG. 1. SEM microphotographs and EDS results of hydrogel modified with 10% HAp and 0,2%GO after incubation in SBF

Conclusions

Among the materials tested, the most promising results were obtained for the hydrogel modified with 10% HAp + 0.2% GO. The resulting composites exhibit mechanical properties similar to those of natural cartilage as well as a significant improvement in thermal properties (compared to sodium alginate) and a small change in pH during incubation in PBS and Ringer fluid.

Based on the obtained results it can be concluded that the hydrogels modified with GO and HAp possess some promising potential for the regeneration of cartilage tissue.

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EVOLUTION AND APPLICATION OF NEW GENETIC TECHNIQUES - FROM NUCLEIC ACIDS VIA MICROARRAYS TO CRISPR

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[ENGINEERING OF BIOMATERIALS 148 (2018) 80]

Introduction
Analysis of human chromosomes has evolved over the last 130 years. The development of chromosomal banding techniques in the 1970s marked the start of a period of innovation in cytogenetics which most recently has seen microarrays (aCGH) being used to investigate copy number variation.

Results
With the advent of aCGH technology which happened several years ago, genetic testing has been more comprehensive and precise. Although next generation sequencing makes fast progress and seems to slowly replacing aCGH, this technology can still produce very useful molecular genetic data and it is a great tool for diagnosing human diseases. The work presents techniques used in the Genetics Departments of Medical University in Lublin for the diagnosis of genetic diseases, including cancer.

Conclusions
Lately, we explore how genetic techniques may serve as the basis for development of future diagnostics tests and therapeutic agents.

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INITIAL IN VIVO BIOCOMPATYBILITY EVALUATION OF BIOMATERIALS USED IN THE NEW BIOMIMETIC HEART VALVE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 81]

Introduction

The new polyurethane valves were designed for extracorporeal ventricular assist device ReligaHeart® EXT in order to guarantee athrombogenic construction, by reduction of turbulence and sufficient valve wall washing [1,2].

The single-leaflet inflow valve and double-leaflet outflow valve prototypes, consisting of very thin titanium – polyurethane composite flexible leaflets were manufactured. The titanium surface was modified with a-C:H carbon surface layer to improve the surface mechanical, physical and biological properties.

After first biomaterial blood compatibility in vitro evaluation [3], the initial in vivo biocompatibility investigation was performed.

Materials and Methods

Titanium Grade 2 surface was modified with a-C:H carbon layer with the thickness of 110nm using magnetron sputtering. Biomaterial flat samples, 10mm diameter and 1,5mm thick, sterilized with ETO, were initially investigated according to biocompatibility standard for medical devices, including tests for: irritation and skin sensitization (ISO 10993-10), local effects after implantation (ISO 10993-6) and systemic toxicity (ISO 10993-11). All in vivo tests were performed with the utilization of New Zealand white rabbits, both sexes, weighing more than 2 kg.

In the irritation and skin sensitization test the biomaterial extracts were injected in animals (n = 3). Directly after injection and then after 24, 48 and 72 hours the detail evaluation of animal skin was performed in order to detect edema or erythema - signs of skin irritation.

In the local effects and systemic toxicity after implantation tests 6 animals had been subcutaneously biomaterial implanted (2 implants for every animal) as well as 6 animals as control group had been operated (only surgical procedure, no biomaterial implanted). The observation period included 28 days. Every day the post-operative scar macroscopic evaluation was carried out (healing level, tissue status around the implant location). General animal behaviour and condition were observed. Before the implantation as well as before euthanasia the blood samples were collected for haematological and biochemical evaluation; the animals were weight. The macroscopic evaluation of post-operative scar and tissues around biomaterial implants were done. After the experiment vitals samples (heart, thymus, liver, spleen, kidneys and lungs) were collected for histopathological examination.

Results and Discussion

a-C:H carbon layer on Titanium Grade 2 surface extract did not induce any skin irritation. Biomaterial implantation revealed no signs of abnormal local inflammatory reaction as well as no signs of systemic toxicity. No statistically significant difference was observed for any tested haematological and biochemical parameters.

Clinical signs - no mortality, behavioural changes, treatment-related adverse clinical signs or signs of physical self-mutilation indicating localized or neurological toxicity were observed during the post-operative examinations, or at the time of euthanasia in any of the groups.

Body weights - all the groups showed gradually weight increasing related to animals' feeding.

Food consumption - all groups showed normal food consumption initially following the postsurgical period.

Histological studies after subcutaneous biomaterial implantation in rabbits showed no pathological changes in the adjacent to the implant tissues and in other organs.

Conclusions

Standardized tests to detect skin sensitization and irritation, local tissue reaction and systemic toxicity, demonstrated the safety and biocompatibility of evaluated a-C:H carbon layer on Titanium Grade 2 surface, in accordance with the ISO-10993 requirements.

The performed studies together with in vitro biocompatibility evaluation carried out before, confirmed that the biomaterial can be safety used as a construction material for the new biomimetic heart valve in extracorporeal heart prostheses for the period of 28 days. Further biological investigation of ReligaHeart® EXT devices equipped with the new polyurethane valves will be continued as well as biocompatibility study will be complemented for long-term clinical utilisation.

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INITIAL IN VIVO BIOCOMPATYBILITY EVALUATION OF MODIFIED Ti6Al7Nb ALLOY SURFACE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 82]

Introduction
In the clinical version of Polish implantable rotary blood pump ReligaHeart® ROT [1] the advanced surface engineering technology was applied for titanium blood pump mechanical and biological improvement. Additionally, blood pump rotor elements manufactured from Ti6Al7Nb alloy could have accidental contact with ceramic composite ZrO_2-Y_2O_3 elements of pump housing. Due to the high hardness of ceramic it is crucial to improve the titanium surface wear resistance.
Modification of the well-known glow discharge assisted nitriding process called as active screen plasma nitriding has been used to produce of TiN+Ti_2N+αTi(N) diffusive surface layers, confirmed as high corrosion and wear resistant [2] as well as biocompatible at in vitro examinations [3].
The initial in vivo biocompatibility investigation was performed as the part of normative required preclinical ReligaHeart® ROT device evaluation.

Materials and Methods
The athrombogenic diffusive nitrided surface layers TiN+Ti_2N+αTi(N)- type have been produced on Ti6Al7Nb titanium alloy surface, with roughness of Ra=80nm, using plasma nitriding process with active screen. Biomaterials flat samples of TiN+Ti_2N+αTi(N) and reference titanium alloy Ti6Al7Nb (14mm Ø) and ZrO_2-Y_2O_3 (8mm Ø), all 1,5mm thick, were sterilized with ETO as the final device ReligaHeart® ROT sterilisation method (EOGas 4, H.W.Andersen Products Ltd.).
The initial in vivo investigation was performed according to biocompatibility standard for medical devices, including tests for: irritation and skin sensitization (ISO 10993-10), local effects after implantation (ISO 10993-6) and systemic toxicity (ISO 10993-11).
The skin sensitization test was performed by the closed-patch test (Buehler method). Healthy adult albino genuine pigs (n = 51) of either sex where utilized (17 animals for each material including 12 tests and 5 control animals). After the induction phase (3 weeks) and next challenge phase (36h) the animal skin was inspected to evaluate the erythema and/or swelling occurrence, in accordance with Magnusson and Klingman scale (MKSc).
Local effects and systemic toxicity after implantation tests were carried out with the utilization of New Zealand white rabbits (n = 32), both sexes, weighing more than 2kg. The 8 animals had been biomaterial subcutaneous implanted (4 implants of Ti6Al7Nb and TiN+Ti_2N+αTi(N), 2 implants of ZrO_2-Y_2O_3 for each animal) and 8 animals as control group were treated (only surgical procedure, no biomaterial implanted). Biomaterials dosage were calculated in order to select the proper sample mass comparing to animal mass, to simulate biomaterial mass used in blood pump recalculated for 1 kg of human body. The initial observation period was 12 months. Every day the post-operative scar macroscopic evaluation was carried out (healing level, tissue status around the implant location). General animal behaviour and condition were observed. Before the implantation as well as before euthanasia the blood was collected for haematological and biochemical evaluation and the animals were weight. The macroscopic evaluation of post-operative scar and tissues around biomaterial implants were done. After the experiment vitals samples (heart, thymus, liver, spleen, kidneys and lungs) were collected for histopathological examination.

Results and Discussion
Standardized tests to detect skin sensitization, local tissue reaction and systemic toxicity, demonstrated the safety and biocompatibility of evaluated TiN+Ti_2N+αTi(N) diffusive surface layers as well as ceramic composite ZrO_2-Y_2O_3, in accordance with the ISO-10993 requirements.
No statistically significant difference was observed for any tested parameters, white blood cell (WBC), C Reactive Protein (CRP), before implantation, and 12 weeks post-implantation.
Clinical signs - no mortality, behavioural changes, treatment-related adverse clinical signs or signs of physical self-mutilation indicating localized or neurological toxicity were observed during the post-operative examinations, or at the time of euthanasia in any of the groups.
Body weights - all the groups showed gradually weight increasing related to animals' feeding
Food consumption - all groups showed an increase in food consumption initially following the postsurgical period, and then food consumption levelled out.
No signs of swelling or erythema was observed on animals skin after contact with investigated biomaterials. The biomaterials did not induce any skin sensitization in allergic reaction of animals (value 0 regarding MKSc). Both biomaterials were classified as a non-sensitizer within Buehler method performed according to EN ISO 10993-10 standard.

Conclusions
The performed studies together with in vitro biocompatibility evaluation carried out before, confirmed that the biomaterial can be safety used as a construction material for the implantable blood pump for the period of 12 months.
No allergic reaction was observed for TiN manufactured on TiAl6Nb alloy as well as ZrO_2-Y_2O.
Further long-term biological and durability study investigation of ReligaHeart® ROT device will be continued in order to allow the device for clinical utilisation in long-term heart support.

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

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THE IMPACT OF UNMODIFIED GRAPHENE - BASED SUBSTRATES ON BASIC PROPERTIES OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS *IN VITRO*

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[ENGINEERING OF BIOMATERIALS 148 (2018) 83]

Introduction
Stem cells (SCs) are a unique type of cells with high self-renewal potential and the ability to differentiate into specialized cell types that build human tissues and organs. One of the most promising groups of stem cells are human umbilical cord-derived mesenchymal stem cells (hUC-MSCs). hUC-MSCs are distinguished by high proliferative potential, the ability to differentiate into many cell types such as chondrocytes, osteocytes or adipocytes, and a non-invasive method of obtaining [1]. Therefore, hUC-MSCs are a promising therapeutic tool in regenerative medicine and tissue engineering.
In order to effectively use the potential of hUC-MSC for differentiation and tissue regeneration, new biocompatible scaffolds for cell culture are still being sought for. An interesting material for biomedical applications due to its unique physicochemical properties is graphene and its derivatives [2, 3].
The main goal of this study was to investigate the impact of unmodified graphene-based substrates on the morphology, proliferation and viability of hUC-MSCs.

Materials and Methods
Various types of solvents (water, ethanol) and thickness of the graphene layer were tested in the studies. After 72 hours of cell culture of graphene surface we investigated the effect of pure graphene and graphene with the addition of surfactant (Pluronic PE 6400) on the morphology, proliferative capacity and cell viability of hUC-MSCs. The proliferation rate was evaluated using a cell counter. Cell viability and apoptosis were measured by using FITC Annexin V Apoptosis Detection Kit and analyzed by flow cytometer.

Results and Discussion
Obtained results revealed that the tested unmodified graphene - based substrates reduce the proliferation and viability of hUC-MSCs compared with control conditions (TC-treated plastic). Cell proliferation and viability decreased with increasing thickness of the graphene layer. The most preferred cell scaffold for cell viability was pure graphene prepared in ethanol solvent.

Conclusions
The presented results indicate the need for further research for the biofunctionalization of unmodified graphene-based substrates, which would increase their biocompatibility for stem cells and enable their biomedical application.

Acknowledgments
This study was funded by StrategMed 3 (NCBiR) and Symfonia 3 (NCN) grants.

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MECHANICAL PROPERTIES OF POROUS COLLAGEN/ GELATIN/ HYDROXYETHYL CELLULOSE MATRICES CONTAINING MICROSPHERES BASED ON SODIUM ALGINATE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 84]

Introduction

Microencapsulation may be defined as a process to entrap one substance within another substance for the purpose of shielding the active ingredient from the surrounding environment. [1,2]. Therefore, this technique is mainly used in areas where particular attention is paid to the stability, efficiency and bioactivity of the obtained materials and it is used, among others things, in the pharmaceutical, medical, food and cosmetics industries [3].

The aim of this paper is to obtain and characterize the mechanical properties of composite materials for potential dermatological applications. The materials were prepared by incorporating polymer microparticles (containing pot marigold (*Calendula officinalis*) flower extract) in the three-dimensional polymer matrix with a porous structure.

The microspheres were produced from sodium alginate and sodium alginate with addition of starch through the emulsion and extrusion method. Alginates are salts of alginic acid and they're extracted from the cell walls of brown algae (*Phaeophyceae*). The natural origin of sodium alginate makes it a very attractive ingredient due to its characteristics, such as: non-toxicity and biodegradability [4]

The obtained microspheres were incorporated into a collagen/gelatin/hydroxyethyl cellulose matrix.

Materials and Methods

Sodium alginate-based microspheres were prepared by extrusion and water-in-oil emulsion described by Muthukumarasamy et al. with modifications [5]. Sodium alginate and starch were used as wall materials for microencapsulation of *Calendula officinalis* flower extract.

Extrusion was performed by expression of the wall material/*Calendula officinalis* flower extract mixture through a syringe needle (⌀=0.6mm) dropwise into 0.5 M $CaCl_2$. Microspheres were held at room temperature for 30 min to ensure complete solidification and then they were separated by filtration.

The emulsification (phase separation) method involved using paraffin oil containing tween 80. The mixture was stirred at 800 rpm for 5 min to form a uniform water-in-oil emulsion. Then 0.5 M $CaCl_2$ was added at the sides of the beaker until the emulsion was completely broken. The beads formed were collected by vacuum filtration.

Collagen (col) type I was obtained in our laboratory from fish scales of *Esox lucius* [6]. Gelatin (gel)/ hydroxyethyl cellulose (hec)/ col matrices were prepared as described in our earlier paper [7]. The weight ratio of collagen and gelatin to microspheres was 1:15.

The mechanical testing was carried out using a mechanical testing machine (Z.05, Zwick/Roell, Germany) at room temperature. The presented values are the average values calculated from five measurements for each type of matrix.

Results and Discussion

Porous collagen/ gelatin/ hydroxyethyl cellulose - based composites were fabricated by using freeze-drying method. The microspheres obtained by extrusion method were significantly larger than the microspheres obtained by emulsion method and the size of the incorporated particles affects the stiffness of the obtained materials (TABLE 1). In addition, the composition of the wall material plays an important role.

TABLE 1. Compressive modulus of col/gel/hec matrices with incorporated sodium alginate-based microspheres

The type of sample/microspheres	Ec [kPa]	
	extrusion method	emulsification method
CONTROL SAMPLE	6.10 ± 0.10	
SODIUM ALGINATE	5.95±0.25	7.62±0.38
SODIUM ALGINATE/STARCH	6.76±0.23	6.72±0.45

Conclusions

During the designing of polymeric materials containing incorporated microspheres, we can influence their mechanical parameters by adjusting the size of the used microparticles and their composition. We developed a novel delivery system for controlled released of a pot marigold flower extract that might be helpful in treating chronic skin wounds or in other dermatological and cosmetic applications. Further studies are required to evaluation of release profile of *Calendula officinalis* flower extract.

Acknowledgments

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CYCLODEXTRINES AS AN MODIFYING AGENT FOR PROTEIN MATRICES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 85]

Introduction
Cyclodextrins (CD) are a cyclic oligosaccharides consisting of 6, 7 or 8 glucose units. The characteristic distribution of hydroxyl groups causes that the cyclodextrins have a hydrophobic interior and a hydrophilic outer part. This makes them a useful chelating molecules that can form inclusion complexes with various compounds [1-2]. Due to the presence of polar groups on the outside of the cyclodextrin ring, they can also interact ionically with biopolymers and stabilize polymer network.
The aim of our work was to investigate the influence of β-cyclodextrins addition on protein hydrogel properties.

Materials and Methods
The collagen was extracted in our laboratory from rat tail tendons and elastin from porcine aortas. The blends containing collagen and 5% and 10% of elastin were prepared. The 5% and 10% of β-cyclodextrin (Sigma-Aldrich) was add to protein solutions. The mixtures were incubated 30 min on magnetic stirrer and then dialyzed against deionized water.

Results and Discussion
The addition of β-cyclodextrin slightly improve mechanical properties of collagen/elastin hydrogels, when 5% of CD is used (TABLE 1). It is surprising, that the temperature of the second step of thermal degradation decrease for collagen gel and increase for collagen/elastin blends with CD addition. In all cases, the weight loss is lower after modification. Moreover, there was no negative effect of CD addition on cell viability on collagen/elastin hydrogels.

TABLE 1. Young's Modulus and parameters of the second step of thermal degradation of collagen/elastin gel modified by CD addition.

		Thermal degradation	
Sample	**E [kPa]**	**Temp [°C]**	**Weight loss [%]**
Coll	8.04±2.93	322	62.3
Coll-5CD	9.66±2.92	319	61.0
Coll-10CD	9.48±2.88	320	54.6
95Coll-5El	7.95±3.03	317	64.0
95Coll-5El-5CD	10.71±3.34	323	62.5
95Coll-5El-10CD	7.94±2.89	323	62.6
90Coll-10El	6.99±2.22	321	64.6
90Coll-10El-5CD	7.11±2.81	323	61.8
90Coll-10El-10CD	5.32±1.60	314	62.2

Conclusions
The ionic interactions between CD and collagen and elastin slightly improve hydrogels mechanical properties. The gels containing 5% CD are stiffer than materials with higher CD addition. The increase of the temperature of thermal degradation of collagen/elastin blends is also observed. It would be caused by the presence of higher amount positively charged functional groups in elastin than in collagen. The materials modified by CD are well tolerated by 3T3 cells.

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POTENTIAL STERILIZATION METHODS FOR IMPLANTABLE DEVICE FOR THERAPEUTIC DELIVERY

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[ENGINEERING OF BIOMATERIALS 148 (2018) 86]

Introduction

Surgical implants intended to deliver therapeutics should fulfil essential requirements, such as mechanical and chemical stability in physiological environment during treatment of a disease or during their lifespan, and then be explanted or gradually degraded. In order to guarantee proper functioning of such system, especially in the case of complex configuration or multifunctional tasks of the implant, e.g. active medical devices, besides selection of biomaterials and involved manufacturing processes, the designer should also consider potential sterilization method. Academia scientist involved in early stage development of a system delivering therapeutics often ignore possible detrimental effect of sterilization on material properties or functioning of the device. Though the sterilization by a validated method is critically needed when it comes to commercialization of the new device.

Validation of selected sterilization technique in terms of its effectiveness, reliability and reproducibility is the prerequisite the manufacturer is requested to demonstrate to the notifying authorities in order to prove microbiological safety of the new device.[1]

The manufacturer intended to distribute medical products within EU should follow regulations specified in directives of 90/385/EEC, 93/42/EEC, 98/79/EC, updated with Regulation (EU) 2017/745 of April 5th 2017, with regards to sterilization, and further, may follow the guidance provided in ISO standards. Reduction of the bioburden on and in the device to Sterility Assurance Level (SAL) 10^{-6} is required.

Materials and Methods

The theoretical approach to selection of potentially applicable sterilization methods is based on the knowledge of properties of the polymeric materials composed the device of bio-electronic implant intended for delivering therapeutics from genetically engineered cells stimulated by light, the complexity of its design and presence of sensitive components or subsystems, FIG. 1.[2] The device should be provided sterile for cells loading, therefore terminal sterilization of manufactured implant or aseptic processing of pre-sterilized components may be applied.

Results and Discussion

Approach to selection of a sterilization technique should begin on screening materials, components or systems included in the implant device. If all of these can withstand high temperature, a dry hot air (160-200°C) or moisturised air (steam, 121 or 134°C) methods may be the first choice. The methods are very reliable and easy to control. The complex shapes and inner elements are also heated, thus the device is entirely sterilized. The method is in principle restricted for thermoplastics, and unsuitable for biodegradable polymers. However, high degree of crystallinity of polymeric materials with high melting temperature may reduce potential negative effects of thermal treatment. Encompassed electronics as well as the presence of optically functional polymers – opacity may occur, eliminate thermal methods of sterilization.

As only low-temperature methods are acceptable, radiation may be considered – its reliable and relatively inexpensive. Either electron beam or gamma rays are highly penetrable and provide sterility of the entire implant, not only its surface, which is especially appropriate for complicated shapes or highly porous materials. Many polymers may be sterilized by radiation, even some biodegradable ones.[3] Nevertheless, the delivered energy may cause polymer degradation, which (if not compensated by crosslinking) reduces applicability of this method. Besides, radiation induces severe deterioration of electronics (one should note that thermal annealing may restore its operation).

Plasma - hydrogen peroxide may be considered, and applied as effective surface sterilization method. One should take under consideration rise of the temperature (40-60°C) and pressure changes during the process. Surfaces not resistant to highly oxidative environment may be altered; this may influence optical properties. In general, it can be applied to electronic systems. Yet, ISO standards have not been developed for plasma method.

The other option is the ethylene oxide sterilization, commonly utilized of polymers and combined materials. The method can be applied for optics and electronics. Beside temperature rise (30-65°C) and rapid pressure changes, a dissolution of the gas (highly toxic) in the polymeric biomaterial and possible chemical reactions with the polymer should be measured.

If the multicomponent implantable system cannot be sterilized by a single method due to incompatibility of various material and components, the common practice is to separately apply different sterilization processes for the individual material/part and assembly the device under aseptic environment (aseptic processing).

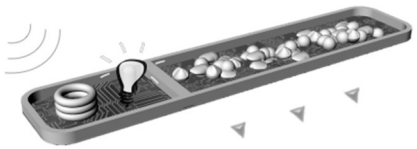

FIG. 1. Concept of wireless-powered cell-based implant for therapeutic delivery.[2]

Conclusions

The course of selection of potentially applicable sterilization methods for developed implantable bio-electronic device for therapeutics delivery was presented. Validation of these methods will be done experimentally, according to specific ISO guidance, evaluating possible alternation of physical and chemical properties of the implant, and followed by biocompatibility and functional assessment.

Acknowledgments

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IN VITRO OXIDATIVE DEGRADATION OF DIBLOCK COPOLYMERS P(LA-b-TMC)

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[ENGINEERING OF BIOMATERIALS 148 (2018) 87]

Introduction

Biodegradable polymers, especially aliphatic polyesters such as polylactide (PLA), poly(ε-caprolactone) (PCL), polyglycolide (PGA), or polytrimethylene carbonate (PTMC), as well as their copolymers have attracted great attention because of their acceptable biocompatibility[1], and have been widely used in various medical applications.[2] PLA is an intrinsically semicrystalline polymer with the glass transition temperature (T_g) about 65°C and relatively high melting temperature (T_m), typically over 170°C. With good tensile strength, low extension, and a high tensile modulus (ca. 3 GPa), PLA has been considered as an ideal biomaterial in the field of load-bearing applications, e.g. as orthopedic implants. Nevertheless, the high crystallinity of PLA results in poor elasticity and long resorption time of 2-3 years.[3] Generally, amorphous poly(1,3-trimethylene carbonate) (PTMC) with T_g at ca. −20°C has excellent flexibility.[4] Copolymerization is usual and efficient method of tailoring polymeric biomaterials properties because the specific architecture and composition of the copolymer can be obtained relatively easily. Therefore, combined properties of a rigid chain from high T_g polyester with a soft segments from rubbery polycarbonate can be achieved by introducing PTMC as a soft block into brittle PLA segments.[5] Such modification is also crucial in terms of biodegradation mechanism and kinetics of discussed copolymers. In this work, we have focused on oxidative degradation of P(LA-b-TMC) copolymers with lactide (LA) contents 70% and 50%, since the data on that are unavailable in the literature.

Materials and Methods

Samples of P(LA-b-TMC) block copolymers with compositions LA/TMC 50/50 and 70/30 were subjected to oxidative degradation. As controls, homopolymers of PLA and PTMC were used. Rectangular specimens of approximately 50 × 5 × 0.3 mm were incubated in 3% hydrogen peroxide solutions with 60 mM Co^{2+} at 37°C, refreshed once a week. Samples were periodically removed from the incubation solution, washed with deionized water and vacuum dried at room temperature to constant weight. Samples were subsequently investigated by physicochemical methods. Tensile properties were examined and these results were complemented by evaluation of mass loss, water uptake, contact angle, morphology (SEM), molecular weight of polymer (GPC) and its thermal properties (DSC). Data are discussed in comparison with ^{1}H NMR results which allow to follow changes of the composition and average sequence distribution of LA/TMC components.

Results and Discussion

During the first 5 weeks, all samples exhibit negligible weight loss of less than 0.5% and very similar degradation rate based on the GPC results. Thereafter, the degradation rate becomes different for samples with various LA contents. For PLA and samples of LA/TMC molar ratio 70/30 a small weight loss below 1.0% is detected at 15 weeks. In the case of P(LA-b-TMC) 50/50 and PTMC, apparent weight loss is detected from 10 weeks and steadily increases to 2.0% and 5.0% at 15 weeks, respectively. In contrast to the weight loss, the molecular weight of PTMC sample and P(LA-b-TMC) 50/50 sample remained constant for 10 weeks, further a slight decrease was observed. In the case of PLA and P(LA-b-TMC) 70/30 samples a substantial drop in molecular weight was observed from 5 weeks, which was reflected in mechanical properties of the studied materials. The compositions of investigated polymers remain almost unchanged during the first 5 weeks, as evaluated with NMR, that is in agreement with the fact that no significant mass loss was detected. Beyond that time, LA content decreases from 70% to 66% for P(LA-b-TMC) 70/30, and from 50% to 40% for P(LA-b-TMC) 50/50. These findings indicate that LA units are preferentially degraded in copolymers with higher contents of TMC. The compositional changes indicated that TMC units are degradable in P(LA-b-TMC) 50/50 copolymers although PTMC homopolymer is not degradable.

PLA and P(LA-b-TMC) 70/30 samples were becoming brittle and fell apart into pieces during degradation. PTMC and P(LA-b-TMC) 50/50 samples deformed – attained globular shape, became soft, and adhered to vessel wall. The original samples exhibited a smooth surface, however in the course of degradation their surfaces became rough and a highly hollow structure was detected after 5 weeks, the size and depth of the hollows increased with the incubation time.

Conclusions

In this study, we have demonstrated that homopolymers and their copolymers respond differently to oxidative degradation, depending on the morphological and chemical composition of the material. PTMC is more prone to oxidative degradation as compared to P(TMC-b-LA) 50/50. Given this knowledge on degradation characteristics of different materials, we are able to tailor degradation characteristics by combining PLA backbones with additional PTMC. The toolbox of techniques that has been used to study the degradation of biomaterials can be applied and employed to screen, limit and select biomaterials that are going to be used for pre-clinical in vivo studies with regard to a variety of clinical applications.

Acknowledgments

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SODIUM HYALURONATE/ GRAPHENE OXIDE HYDROGELS FOR CARTILAGE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 88]

Introduction

Conventional methods of cartilage regeneration are less effective than tissue engineering approaches because of avascular character of a cartilage and its hierarchical structure. Considering a biomimetic approach and intrinsic characteristic of a cartilage, gradient hydrogel scaffolds seem to be a promising solution. Among many available materials, sodium hyaluronate (HA) as an essential extracellular matrix (ECM) component is particularly interesting for the design and development of tissue engineering scaffolds. This naturally occurring anionic polysaccharide is well-known for its biocompatibility, biodegradation and superior biological properties. By introducing appropriate modifying phases and thus creating composite systems they can be further improved[1-5]. Addition of graphene oxide or reduced graphene oxide can significantly improve structural, thermal, surface, and mechanical properties of polysaccharide-based composites[6]. Hence, in the current study advantages of sodium hyaluronate and graphen oxide were combined to obtain gradient scaffolds for cartilage tissue engineering.

Materials and Methods

The aim of this research was to fabricate composites based on sodium hyaluronate (HA, 1% w/v of water) (Acros Organics) modified with different contents of graphene oxide (GO, 0-3% w/w of HA) (ITME, Poland). Tannic acid (TAc, 50% w/w of HA) (Acros Organics) and iron (II) chloride ($FeCl_2$, 10% w/v) were used as crosslinking agents. The samples were prepared as films by solvent-casting into Petri dishes and as porous scaffolds by freeze-drying (-80°C) in 24-well plates (FIG. 1). Crosslinked samples were obtained by immersing in $FeCl_2$ solution for 72 h (FIG. 2). Microstructural (digital microscope, SEM), structural (FTIR-ATR), mechanical (static tensile and compression tests) and surface (wettability) properties of the obtained materials were evaluated.

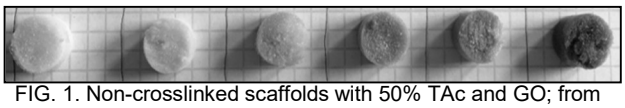

FIG. 1. Non-crosslinked scaffolds with 50% TAc and GO; from left: 0.1; 0.2; 0.5; 1; 1,5; 3 %

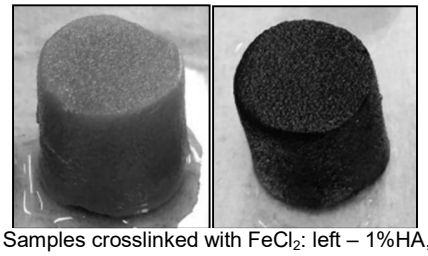

FIG. 2. Samples crosslinked with $FeCl_2$: left – 1%HA, right – 1%HA/50%TAc/15%GO

Results and Discussion

Firstly, screening was done to find the optimal concentration of TAc (a physical crosslinker and an antioxidant agent). It was observed that the addition of tannic acid improves the mechanical properties (FIG. 3) and the optimal amount of TAc was found to be 50% w/w of HA. When combined with $FeCl_2$ as a chemical crosslinker, a stable and elastic structure was obtained. Moreover, $FeCl_2$ had beneficial effect on the wettability of the materials. Addition of GO improved porosity and mechanical properties. The more GO was introduced, the higher the tensile strength of films and compressive strength and Young's modulus of the porous scaffolds were. Its simultaneous addition allowed to customize the properties.

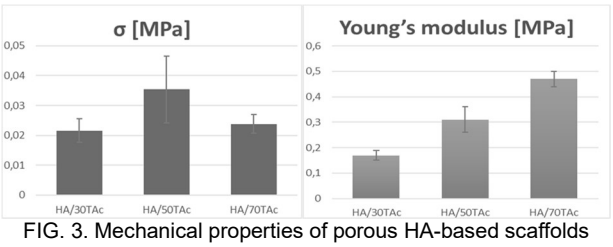

FIG. 3. Mechanical properties of porous HA-based scaffolds crosslinked with TAc (compression test).

Conclusions

In the proposed multiphase hydrogel systems, beneficial properties of sodium hyaluronate, graphene oxide, tannic acid and iron chloride (II) were combined. Hierarchical structure was created, that consists a potential for cartilage tissue regeneration, but further studies are necessary to investigate e.g. biological properties.

Acknowledgments

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COMPOSITE MATERIAL SELECTION FOR A PATIENT TRANSFER ASSIST DEVICE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 89]

Introduction

Laminates - layered composites containing at least two elements (matrix and reinforcement) widely used in industry have also found their usage in medicine. Their main feature is the ability to modify the material in order to obtain the required properties. Depending on the needs, we can modify reinforcement, type of resin or the method of bonding substrates. Commonly used fibers are: carbon fiber, glass and aramid fibers; resins are epoxy, vinyl and polyester. The following commercial forms of fibers are distinguished: roving, mat and fabric (multidirectional and unidirectional - UDC) with several styles of bandwidth connections: differing in number of layers and the way they are combined (at various angles or in different ways of sewing/ plaiting) [1, 2] (TABLE 1).

TABLE 1. Comparison of mechanical properties of carbon, glass and aramid fibers [1,2].

Fiber	Density ρ [g/cm^3]	Tensil strength Rm [MPa]	Young's Modulus E [GPa]
Glass	2,5-2,6	1350-4900	60-90
Carbon	1,6-2,0	2800-5490	230-588
Aramid	1,44-1,47	2900-3450	59-179

The main goal of this project is to select a proper composite material for a medical device – lightweight, durable and ergonomic patient lift which efficiently transfers patients between two plains. The material selection contains the choice of matrix, reinforcement, form of fibres, style of bandwidth, the thickness and the fibre mass for the individual layers of laminates, as well as the strength calculation.

Materials and Methods

Based on a comparison of composite material properties, an epoxy-carbon laminate was selected. High stiffness and low weight have outweighed the lower durability for dynamic loads that are not present in the design. The proposed laminate structure is made of three layers arranged alternately. The outer layers will be a multidirectional fabric in the Biaxial (BXC) version, in which the carbon fibers are laid at an angle of +/- 45º, while the inner layer will be the UDC roving fabric with carbon fibers arranged in one-directional manner. A literature review allowed to construct the following assumptions: BXC type outer layers should have an equal thickness and the ratio of the thickness of the inner layer to the outside should be approximately 2:1.
In order to carry out strength calculations, the shape and size of device was designed using AutoCAD.
The strength calculations assumed lifting capacity of 150 kg and were performed in the following order: determination of elementary stresses, determination of the most dangerous cross-section, calculation of tensile strength, calculation of Young's modulus, selection of channel dimensions, determination of material parameters (thickness, fibre mass), determination of substitute stresses. The calculations regarding the properties of laminates have been made in accordance with the European standard EN ISO 12215-5: 2008 [3].

Results and Discussion

Lamination technology was one of the criteria that determined the shape of the profile. A contact method is proposed for applying laminate layers to a specially prepared internal form. A profile in a C-shape and dimension of 138x90x14 mm has been designed. Patient lift includes an arm of 1,6m height and 1m length of the horizontal beam (FIG. 1).

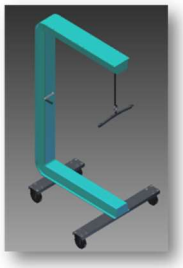

FIG. 1. 3D Model of the proposed patient transfer assist device.

The vertical bar is simultaneously bent and compressed, while the horizontal bar is bent and sheared. Using the mathematical formulas contained in the EN ISO 12215-5: 2008 standard, the results of the main strength calculations have been presented in TABLE 2.

TABLE 2. The main calculations of a C-shaped patient lift designed with carbon-fibres [3].

TENSIL STRENGTH Rm [Pa]	YOUNG' S MODULUS E [Pa]	ALLOWABLE STRESSES k [Pa]	STRESS σ_z [Pa]		DEFLECTION f_c [m]	
			Vertical	Horizontal	Vertical	Horizontal
491526000	49740000000	163842000	7958879	1742431	0,007504	0,003312

The profile meets the strength conditions ($\sigma_z \leq k$) and the deflection (f) is acceptable. Selection of the C-profile dimensions allowed also to determine the thickness and the fibre mass for the individual layers of laminates (TABLE 3).

TABLE 3. The mass of carbon fibres of individual layers depending on the mass content of fibres in the laminate and the thickness of the given layer [3].

LAYERS	THE MASS OF CARBON FIBRES w $\left[\frac{kg}{m^2}\right]$	MASS CONTENT OF FIBRES IN THE LAMINATE μ	THICKNESS t [mm]
BXC	0,0033	0,594	3,712963
UDC	0,0056	0,5742	6,571694
BXC	0,0033	0,594	3,712963
Sum	0,0122	0,584911	13,99762

Conclusions

To design a lightweight, durable and ergonomic patient lift with lifting capacity of 150 kg an epoxy-carbon laminate composed of three layers should be used. External BXC layers, each with a thickness of 3.71 mm containing 0.0033 [kg/m^2] of fibre mass and an internal layer of UDC with a thickness of 6.57 mm and a fibre mass equal to 0.0056 [kg/m^2].

Acknowledgments

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3D BIOPRINTING – DETERMINATION OF HYDROGEL BIOINKS PROPERTIES FOR A PRINTER WITH DEDICATED CONSTRUCTION

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[ENGINEERING OF BIOMATERIALS 148 (2018) 90]

Introduction
Biofabrication technology is defined as the production of complex living and nonliving biological microtissues from raw materials such as living cells, molecules, extracellular matrices and biomaterials [1]. Biofabrication is an area of tissue engineering where many solutions can be developed using additive manufacturing (AM), also known as 3D printing (layer-by-layer material deposition). Three different biofabrication approaches can be distinguished – scaffold-based tissue engineering, scaffold-free tissue engineering or bioprinting [2]. Bioprinting is an emerging field that makes a revolutionary impact on medical sciences.
Current medical procedures aim to restore tissue function to patients with diseased or damaged tissues through tissue transplantation and implants. In tissue engineering scaffolds provide an optimum environment or housing for cell attachment and growth, tissue regeneration, fluid movement, and structural integrity. Adaptation of 3D printing into tissue engineering brings unique capabilities in rapid fabrication of tissue scaffolds with controlled porosity and internal architecture, tunable mechanical and structural properties.
Due to its individuation and controllable properties, the ability to print with patient's cells but also from reproducibility, high resolution in microscale production, in the future, bioprinting may develop into a potential tool for organ regeneration and have promising applications in tissue engineering.
Bioprinting allows for the fabrication of 3D tissue constructs with pre-programmed structures and geometries containing biomaterials and/or living cells (together termed the bioink) by synchronizing the bioink deposition/cross-linking with the motorized stage movement [3]. Various 3D printing methods can be used for bioprinting: inkjet (droplet), laser-assisted and extrusion-based [4]. The bioprinting modalities develop significantly, nevertheless their applications are limited by the lack of appropriate bioinks, which both need to meet the requirements for bioprinting and have the proper bioactivity of the different cell types. In order to generate tissue constructs with adequate mechanical strength, retain the tissue-matching mechanics, adjust gelation and stabilization to aid the bioprinting of structures with high shape fidelity, biocompatibility and, if necessary, biodegradability, the bioink should possess the desired physiochemical properties (mechanical, rheological, chemical) and biological characteristics [5].

Materials and Methods
In order to talk about an effective bioprinting, we need to choose the right printing material (bioink), AM technique and parameters. In our laboratory the new construction of 3D printer was designed. Such elements as nozzle length and diameter were firstly numerically simulated and then calibrated trough real prints adjusted the applied bioinks (sodium alginate/gelatin hydrogels). Single line and three dimensional lattice prints were performed in order to configure all movement of the printer and to achieve correctness of prints. Various solvents for hydrogels and different crosslinking method were checked. Rheological properties (rheometer), mechanical properties (Young's modulus and compressive strength) and chemical analysis (FTIR) of hydrogels were conducted. Biological response was evaluated using cells lines in order to check the influence of AM method onto the bioink. The biocompatibility was checked conducting the live/dead and XTT tests according to the ISO norm rules (extracts response and direct reaction to the material).

Results and Discussion
Tests showed that the parameters of custom 3D bioprinter have to be adjusted and tested in real use experiments in order to confirm the numerical simulations. Thanks to the simulation and test confirmation the optimal diameter of the nozzle of 0.32 mm was chosen (adjusted to the peristaltic pump and all the printer configuration). The modified extrusion-based printing method, which were applied in this custom 3D bioprinter, with adjusted temperature and cross-linking method, let obtain the highest possible level of reproduction of the correctness of the final prints. The verified hydrogels composition with various solvents and structure analysis showed that bioinks prepared on the basis of media are better materials for cells proliferation. Porous topography of the hydrogels limits the cells proliferation although they biocompatibility was confirmed.

Conclusions
The simulation allowed to set the output parameters and match them to bioink intended for direct printing usage. Development of the 3D bioprinter must be carried out in strictly controlled parameters for the bioink. The temperature, cross linking agent and method, pressure, composition influence the printability and affect compressive strength of the hydrogels. In the second stage all these factors have an impact on topography and thus on the cells viability.

Acknowledgments
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ASSESSMENT OF MECHANICAL AND BIOLOGICAL PROPERTIES OF SODIUM ALGINATE/GELATINE HYDROGELS DEDICATED FOR BIOPRINTING PREPARED WITH THE USE OF VARIOUS SOLVENTS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 91]

Introduction

According to the report [1], the industry of additive manufacturing methods will increase from USD 6.1 trillion in 2016 to USD 21 trillion in 2020 (from use in medicine, it accounts for 11% of the total AM market). 3D printing allow the development of implants, tissue engineering, artificial organs, controlled drug delivery systems as well as planning operations [2]. Intensively developing field of 3D printing is bioprinting – layer by layer printing of biomaterials with living cells in a recommended pattern [3]. The use of bioprinting methods in tissue engineering has many advantages. First of all, it allows to obtain anatomically appropriate tissues based on previously obtained data from medical imaging. It allows to obtain complex geometries with controlled porosity. Due to the significant development of the bioprinting technology, it is possible to print structures with different types of cells. Cells, as well as other biological components, can be precisely placed in accordance with the pattern previously planned [4]. Printing of functional organs is not yet possible. Achieving this goal is possible through further development of methods of bioprinting and, above all hydrogels provide a good environment for the cell's growth and proliferation, appropriate mechanical properties and printability.

Materials and Methods

For the preparation of hydrogels sodium alginate/gelatin in various conversations, two solvents were used: deionized water and Dulbecco's Modified Eagle Medium medium (DMEM, Corning) supplemented with 10% fetal bovine serum (FBS,Corning) and 1% vol. antibiotics penicillin/streptomycin P/S (Corning). Hydrogels were evaluated for chemical analysis (FTIR), mechanical properties (Young's modulus and compressive strength) and biological response using the EA.hy 926 cell line (ATTC). Hydrogels were tested for stability over time by placing them in a PBS buffer (Corning) solution in 37°C. After incubation time (24h, 48h, 7 days, 14 days, 21 days), the chemical and mechanical properties as well as mass loss were investigated. For hydrogels with the best parameters direct bioprinting test were carried out. Living cells were introduced into the hydrogels with the best parameters and direct bioprinting test were carried out on the custom designed and built bioprinter. The temperature of the nozzle was changed during the bioprinting and its impact on the bioink extrudability and cell viability was assessed. Rheological properties of hydrogels were tested on a rheometer at nozzle temperatures during bioprinting.

Results and Discussion

Regardless of the choice of solvent and the percentage content of sodium alginate and gelatin, the prepared hydrogels do not induce a cytotoxic effect in the reaction with EA.hy926 cells line. Cell adhesion and proliferation depends on the topography of the surface of the prepared hydrogels. The use of a culture medium as a solvent promotes cell proliferation.
In the case of mechanical properties, the use of various solvents primarily affects compressive strength - higher values for hydrogels prepared using water as a solvent. Increasing gelatin content increases the value of compressive strength, as already noted in the research carried out by the Giuseppe's team [5]. The method of sample preparation (temperature and mixing time, crosslinking time) has a significant influence on the values of the determined mechanical parameters.
As a result of incubation in a PBS solution at 37 Celsius degrees, the compressive strength values drop, the mass and pH of the buffer change, what indicates that the degradation processes is taking place.

Conclusions

The properties of hydrogels depend on the concentration and the used solvent. The ability of cells to grow further depends on the topography of the surface of the hydrogels. During the bioprinting, it is necessary to select the temperature that ensures the appropriate viscosity of the hydrogel and to limit the shear stress when extruding through the nozzle.
There are many studies carried out using hydrogels of sodium alginate / gelatin prepared using physiological saline [6], deionized water [7], HEPES medium [8] or PBS as the solvents. As a final aim of our work is to use the hydrogels for the direct bioprinting, we took up an investigation on the effect of a various solvent used at the same content of gelatine and sodium alginate for the chemical, mechanical and biological properties of bionks.

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CHITOSAN MICROCAPSULES OBTAINED BY WET METHOD AS POTENTIAL CARRIERS FOR ACTIVE SUBSTANCE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 92]

Introduction

Hydrogel biomaterials are attractive carriers for many active substances in modified drug release systems. Unfortunately, only few such systems have been introduced to the clinical market. The reason for this is a large number of factors affecting the release of the active compound from this type of carrier [1]. The lack of so-called bioavailability results in a lowering of the therapeutic effect and revocation of the tested system from further basic and clinical trials. The response to these problems is the use of biocompatible and biodegradable hydrogel materials based on natural compounds. An example of such a biopolymer is chitosan, which is characterized by biocompatibility, enzymatic degradation under in *vitro/in vivo* conditions, and its degradation products are non-toxic [2]. An additional advantage of chitosan is its hydrophilicity, the multitude of functional groups undergoing controlled reactions (e.g. combining into a carrier-drug system) and the cationic nature of the chain, which allows to combine chitosan with drugs, nucleotides, proteins or peptides. The release kinetics of the active compound from the chitosan carrier are, however, also influenced by a variety of factors. One of them is the type of cross-linking agent, particle size of the closed (encapsulated) active compound in the polymer network space, or the molecular weight of chitosan alone [3]. Chitosan makes also possible to match the form of the carrier to the place and time of drug release. It can be formed and shaped into micro and nanocapsules, micro and nanometer fibers, or coat the other carriers with a chitosan layer (e.g. by electrospraying).

Bearing in mind the above, a series of chitosan microcapsules was obtained in this study, in which gentamycin was additionally introduced. Various conditions for forming capsules have been used: a different composition of the precipitation bath (NaOH or a mixture of NaOH:KCl) and a different cross-linking agent (carrageen/agar).

Materials and Methods

The microcapsules were prepared from low molecular weight chitosan (Sigma-Aldrich), and carrageen and agar (Sigma-Aldrich) were used as the crosslinking agents. A 5% NaOH solution and 0.3 M KCl solution (POCH) were used to prepare the precipitation bath. The chitosan solution was mixed in a weight ratio of 2:1: with 2.5% agar or 2.5% carrageenan and homogenized for 30 minutes on a magnetic stirrer. A 5% gentamycin (Pharma Cosmetics) was introduced into part of the solution. The coagulation process was carried out at 10°C using NaOH precipitant solution or a 1:1 mixture of NaOH:KCl. The obtained capsules were stabilized for 24h at 15°C, and then the excess of coagulation agents were rinsed with water (until it reached a constant conductivity). The prepared capsules were frozen at -80°C for 24 h, and then lyophilized (-50°C/ 0.3 Torr). Microcapsules were characterized for their morphology and composition (SEM / EDS). Structural studies of chitosan-carrageen (KR) and chitosan-agar (AR) systems were carried out using the FTIR-ATR technique. The size was estimated by determining the equivalent diameter of the microcapsule. The possibility of release of active compounds from the porous surface of the capsule was confirmed by monitoring the release of gentamycin (GS) by the ES-ICP method.

Results and Discussion

The size of CS microcapsules depends on the cross-linking agent used: microcapsules with a larger equivalent diameter were obtained in CS-KR (260 μm) systems than in CS-AR (230 μm) systems, where the precipitating factor was NaOH / KCl solution. The crosslinking agent used did not affect the morphology and shape of the microcapsule. The type of coagulation bath did not affect the microcapsules shape too.

The introduction of 5% gentamicin into chitosan led to an increase and change in the shape of microcapsules (from oval to spherical). A similar trend was retained, as in the case of reference materials (CS-AR, CS-KR): larger microcapsules were obtained in CS-KR-GS systems, where the precipitating factor was a mixture of NaOH / KCl solutions (increase from 260 to 290 μm for the CS-KR-GS system and increase from 230 to 250 μm for the CS-AR-GS system). The introduction of gentamicin increased the surface development of microcapsules, and their roughness. The conducted release tests showed that CS-AR-GS and CS-KR-GS systems release the drug faster than the same materials, however precipitated in NaOH solution.

Conclusions

The preliminary results obtained show that properties such as: morphology, size and rate of drug release can be modeled already at the stage of obtaining materials, through the selection of cross-linking agents or coagulation solutions. The fastest release occurs when chitosan is not cross-linked. The stronger crosslinking agent is carrageen compared to agar, thus the release of the drug from the systems enriched with the KR occurs in the slowest way.

Acknowledgments

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HALLOYSITE NANOTUBES AS POTENTIAL CARRIER FOR GENTAMICIN

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[ENGINEERING OF BIOMATERIALS 148 (2018) 93]

Introduction

The special properties of clay minerals, their widespread availability, low cost and biocompatibility in contact with human tissues made them used as carriers in controlled drug delivery systems. One of these minerals is halloysite, which unique tubular structure makes it distinctive from all aluminosilicates. The empty lumen of halloysite nanotubes is an excellent space to locate various compounds inside, such as proteins or drugs. The main goal of the intercalation of the latter inside halloysite nanotubes is to extend their release time from minutes to even a few hours [1]. The mechanisms of the binding of ofloxacin [2], tetracycline and ciprofloxacin [3], belonging to cationic drugs, were investigated among others. The general observation from these scientific papers is that the maximum amount of bound drug is lower than it would be expected from the halloysite CEC [3]. The aim of this work was to investigate the possibilities of using halloysite nanotubes as a carrier for gentamicin. As part of the study, the effect of three parameters of preparation of the Hal-GS hybrids (temperature, mixing time and initial amount of drug) on the process of attaching the drug to halloysite nanotubes was analyzed.

Materials and Methods

Halloysite Dragonite™ HP (Hal) (Applied Minerals) and gentamicin sulfate (GS) (Amara) were used in the research. Samples were prepared at different temperatures (20, 60 and 80°C), varying the mixing time (2, 12 and 24 h) and using different amounts of the drug determined by the cation exchange capacity (CEC) of Hal. The obtained hybrids were investigated by X-ray diffraction (XRD), infrared spectroscopy (FTIR), zeta potential measurement, thermal analysis (DSC/TG), infrared spectroscopy with total internal reflection (FTIR-ATR) and X-ray photoelectron spectroscopy (XPS). Studies on the kinetics of gentamicin sulfate release were performed in buffered saline (PBS) at pH 7.4 by UV-Vis spectroscopy. Selected materials were subjected to antibacterial tests using the certified *Escherichia coli* ATCC 25922 reference strain (Gram-negative bacillus) to assess the effect of the production process and drug intercalation method on the antibacterial properties of Hal-GS hybrids.

Results and Discussion

The XRD results showed no shift of the peaks from halloysite towards higher values of the 2θ angle, which indicates the lack of the effect of incorporating GS into the interlayer spaces of halloysite nanotubes, regardless of the amount of drug in the initial suspension. Analysis of FTIR spectra did not show bands originating from newly formed chemical bonds, which confirms the attachment of drug particles on the surface of the Hal nanotubes by weak intermolecular interactions. Based on measurements of Zeta potential, it can be estimated that the amount of drug accumulated on the surface of the nanotubes depends on the amount of drug in the initial suspension. The addition of the drug changes the value of Zeta Hal potential from negative to positive. DSC/TG measurements indicate a non-thermal GS thermal stabilization in the presence of Hal. Considering the conditions of formation of Hal-GS hybrids, the highest amount of drug was found in conjugates prepared at 60ºC. The release curves (UV-Vis) indicate that the amount of released drug increases with its increasing content in Hal-GS conjugates. Lack of changes in XPS spectra of silicon, aluminum and oxygen indicates the lack of new chemical bonds. The presence of GS confirms the widening of the peak from carbon. A phenomenon of formation of asymmetric zones of bacterial growth inhibition (FIG. 1) around the Hal-GS pellets was observed. The higher GS content in the initial suspension was, the greater the bactericidal effect.

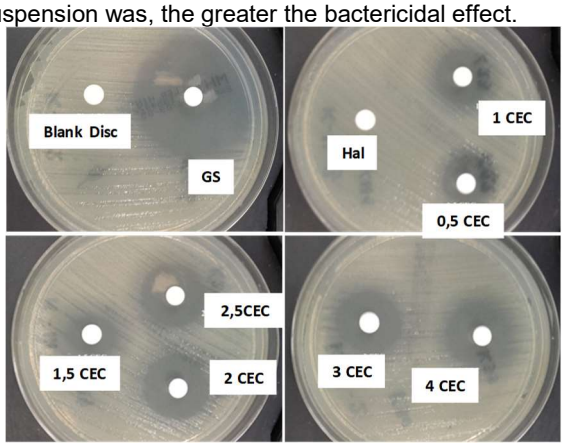

FIG. 1. Hal-GS antibacterial activity.

Conclusions

The results of physicochemical tests indicated that gentamicin sulfate binding occurs only on the surface of nanotubes. The grafting of GS onto halloysite nanotubes is most effective at 60° C with a 24 h mixing time, and the increase in temperature to 80°C causes a dramatic decrease in the efficiency of Hal-GS hybrids formation. The release process of GS from hybrids in the PBS solution proceeded very rapidly, and a maximum time of the drug release measured by spectrophotometry did not exceed 20 minutes. Moreover, the amount of gentamicin sulfate was lower than it would result from CEC measurements, because only the surface of nanotubes was grafted with the drug. The process of producing the hybrids did not affect the antibacterial properties of GS against *E. coli* bacteria. The unusual asymmetry of zones inhibiting the growth of bacteria may lead to the conclusion that the tubular structure of the carrier affects the distribution of the drug in the biological environment.

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INVESTIGATION OF DEPENDENCIES BETWEEN PRINTING PARAMETERS IN ADDITIVE MANUFACTURING AND PROPERTIES OF MATERIALS USED FOR IT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 94]

Introduction

A bioprinting, especially direct bioprinting, gives great hopes and possibilities in the work on natural tissues and organs fabrication. The development of the new materials for additive 3D printing methods gives new possibilities for the bioprinting, but at the same time forces the changes and development of the design of the printers themselves. The continuous development of the 3D printing industry also contributes to the development of bioprinting giving new opportunities. When constructions and materials are developed simultaneously, then the best results are achievable. Therefore, the topic that contributed to the development of materials and devices used in the bioprinting was taken up.

Materials and Methods

In this work optimal parameters of additive printing were developed with selection of proper material parameters of the printed hydrogel. For this purpose, a fully functional 3D printer construction for printing with high viscosity hydrogels was created. The design of the machine took into consideration optimal construction components to achieve best possible results when it comes to quality of the print. To obtain it, nozzle length and diameter were chosen with the use of numerical simulation. What is more, a peristaltic pump to pump hydrogels was designed and manufactured with use of 3D printing techniques. Hydrogel crosslinking method with division into thermal and ionic crosslinking was customized to fit perfectly sodium alginate and gelatin mixture. Moreover, investigations were made to illustrate the capabilities of the constructed machine and the correctness of the printing parameters with chosen material. Configuration tests such as single line and three dimensional lattice prints were performed. Those tests allowed to configure all movement of the printer in order to achieve correctness of prints with the usage of chosen material.

Results and Discussion

In this work both 3D printer construction and parameters of material used for printing were focused on direct bioprinting. In order to make the optimum selection of the nozzle in relation to the planned bioink, numerical simulation of the material flow was carried out by a pre-selected nozzle. The literature indicates a fairly wide range of nozzle diameters used in the bioprinting: from 0.15 mm to 0.55 mm [1]. If a smaller diameter of the needle is used, a high pressure is required to pump the hydrogel. It may cause damage to the cells included in the hydrogel. In the case of a large diameter nozzle, spontaneous flow of material may occur and thus the printout will be losing accuracy. The simulations of the hydrogel flow through capillary allowed to verify and confirm the correctness of the needle with a diameter of 0.32 mm (FIG. 1).

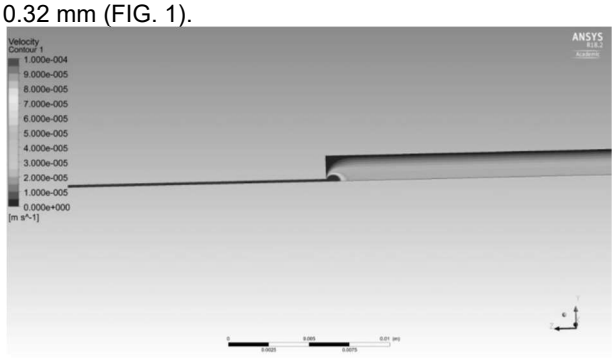

FIG. 4. The result of simulation of flow velocity through the capillary.

A lattice visible on FIG. 2 was printed. Model parameters were set so that each wall was a single print line. This allows for the best observation of individual printed lines, and thus to adjust the optimum print performance.

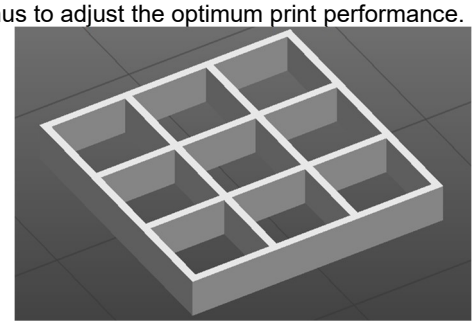

FIG. 5. A multi-layered grid model for testing the accuracy and correctness of spatial prints.

Conclusions

Well determined printing parameters adjusted to the defined material allow to obtain very good printouts on a given device. Worked out custom 3D bioprinter takes into account all the crucial properties of the bioink, which must be included to ensure cells viability before, during and after the direct printing. This system allows direct and indirect (scaffolds) printing for biomedical application.

Acknowledgments

The authors acknowledge dr Joanna Skopińska-Wiśniewska for her valuable and constructive suggestions according to the hydrogels.

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THE EFFECT OF TITANUM DIOXIDE MODIFICATION ON THE COPPER POWDER BACTERICIDAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 95]

Introduction

Copper is known at an important micronutrient required in very small amounts for survival of most aerobic organisms but at high concentrations can become toxic and inhibit microbial growth. Bogdanowic et al. show that Cu NPs (diameter 5,3nm) were able to reduce more than 98% of all tested strain (include *E.coli* and *S.aureus*) after 2 hours of contact and Cu NPs has higher reduction rate for *E.coli* than *S.aureus* [2]. Argueta-Figueroaet al. also confirmed that Cu NPs has the antibacterial activity against *E.coli* and *S.aureus* (MIC - 1000μg/ml) and causa more membrane demages of the *E.coli* as compared of the *S.aureus* [1]. The results of the experiment with another Gram-negative bacteria (*Salmonella*) showed that under dry incubation conditions bacterial cells are extremely vulnerable to copper [6]. He X. et al. also show that CuO/TiO_2 coating has high antibacterial activity against *Staphylococcus aureus* in contrast to pure titanium and TiO_2 coating. It can be explained that the addition of copper is the crucial factor to endow copper/TiO_2 coating with the antibacterial effect against *S.aureus* [4]. The paper proposes modifications of copper powder with amorphous submicron titanium dioxide in order to increase biological activity. The modified powder can be used to create coatings by various methods including thermal methods. The work presents analysis of Cu and TiO_2 powders and results of bactericidal tests carried out on a Cu-TiO_2 composite powder.

Materials and Methods

A dendritic commercial copper powder with a particle size of 50-100 μm obtained by the electrolytic method was used for the tests. The powder was mixed with an amorphous submicron titanium dioxide powder produced by the sol gel method at the Department of Mechanics and Materials Engineering at the Wrocław University of Science and Technology [7]. The powders were mixed in a mechanical stirrer for 4 hours. Four different concentrations of Cu and TiO2 powders were used. The experiment included *Escherichiacoli* ATCC 11775 *and Staphylococcusaureus* ATCC 6538P, strains were used after 24 hours of incubaton in 37°C to prepare the inoculum to a concentration of 10^6cfu/ml. A suspension of *S. aureus* or and *E. coli* was sprayed over the total area of each Petri dish. Then paper discs (7mm diameter) soaked in solution of mixture of Cu-TiO_2 powders (10mg/l) were put on nutrient agar plates. After incubation in 35°C for 48 hours the diameter of inhibition zone was measured.

Results and Discussion

The analysis of the obtained results clearly shows the beneficial effect of the addition of the titanium dioxide fraction on the microbiological activity of the copper powder only against *S.aureus*. The results of zone inhibition method are shown on the Tab. 1 and Tab. 2. Copper NPs and Cu/10%TiO_2 powder show good inhibition zone against *E.coli*. The inhibition zone against *Staphylococcus aureus* was measured for mixed copper and TiO_2 powder, there was no inhibition zone for pure copper and for concentrations of titanium dioxide 10% and 25%. The inhibtion zone of titanium dioxide concentration of 50% was noticed only against *S. aureus* (Gram positive bacteria). The tests confirm the results obtained for TiO_2-Cu coatings produced by magnetron sputtering method where the authors found that addition of copper into titania structure during sputtering process resulted in increasing antimicrobial activity for microrganisms (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Enterococcus hirae* and *Candida albicans*) [8].

TABLE 1. Inhibition zone of Cu-TiO2 powder against E.coli.

Powder composition	Diameter of inhibition zone, mm	
Cu NPs	*E.coli ATCC 11775*	10,5 (3,5)
Cu+10%TiO_2NPs		14 (7)
Cu+25%TiO_2NPs		7 (0)
Cu+50%TiO2NPs		7 (0)

TABLE 2. Inhibition zone of Cu-TiO2 powder against S.aureus.

Powder composition	Diameter of inhibition zone, mm	
Cu NPs	*S.aureus ATCC 6538P*	7 (0)
Cu+10%TiO_2NPs		7 (0)
Cu+25%TiO_2NPs		7 (0)
Cu+50%TiO_2NPs		15 (8)

Conclusions

The studies have shown a positive effect of the addition of TiO_2 on bactericidal properties only against *Staphylococcus ureus* (Gram positive bacteria). A significant increase in the inhibition zone is visible only at the highest tested proportion of the modifying phase of 50% TiO_2. Interestingly, no inhibition zone was found for this strain in pure copper powder tests. The inhibition zone was observed for pure copper powder against *Escherichia coli*. It seems reasonable to develop an optimal concentration of modifying TiO_2 fractions for individual bacterial strains. The modified copper powder can be used as a starting material for the production of coatings using, among others, the cold spray method.

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GRADIENT COPOLYMERS FROM AROMATIC AND ALIPHATIC 2-OXAZOLINES AS PROMISING BIOMEDICAL MATERIALS

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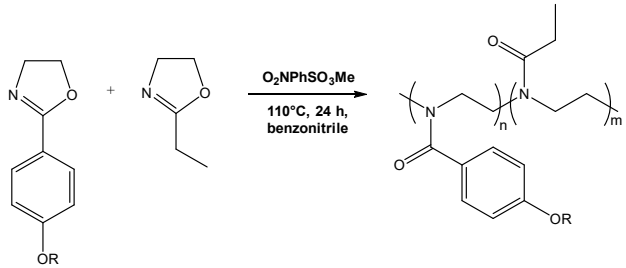
[ENGINEERING OF BIOMATERIALS 148 (2018) 96]

Introduction

Amphiphilic block copolymers play an important role in interfacial and colloid chemistry due to possible to self-assembly processes in solutions or on the surfaces. They play a special role in drug delivery systems as micellar or polymerosome particles encapsulating poorly soluble drugs. Gradient copolymers have been shown as polymeric materials with similar self-assembly properties as in the case of block copolymers. Poly(2-oxazolines) prepared by living cationic ring-opening polymerization (LCROP) represent versatile biomedical polymers with diversity of solubility, functionality, and stimuli responsive behaviour [1]. Block and gradient copolymers from 2-methyl-2-oxazoline and 2-phenyl-2-oxazoline were used for the preparation of stable nanoparticles of different size and particle shape [2]. Recently, we used gradient copolymer of 2-ethyl-2-oxazoline with extremely hydrophobic 2-(4-dodecyloxyphenyl)-2-oxazoline for the encapsulation of curcumin. Prepared nanoparticles exhibited excellent stability, high loading capacity, and efficient cell internalization of encapsulated curcumin [3].
Aim of this contribution is a survey of synthesis, properties and biomedical applications of gradient copolymers of different 2-(4-alkyloxyphenyl)-2-oxazolines with 2-ethyl-2-oxazoline.

Materials and Methods

2-(4-Alkyloxyphenyl)-2-oxazolines were prepared from 2-(4-hydroxyphenyl-2-oxazoline) by the reaction with alkyl halides in the presence of a base. Cationic polymerizations of 2-(4-alkyloxyphenyl)-2-oxazolines with 2-ethyl-2-oxazoline were performed in benzolitrile at 110 C for 24 h using methyl 4-nitrobenzenesulfonate as an initiator (SCHEME 1). Theoretical degree of polymerizations was in all cases equal to 100. Composition of copolymers was determined by NMR spectroscopy.



SCHEME 1. Cationic polymerization of 2-(4-alkyloxyphenyl)-2-oxazolines with 2-ethyl-2-oxazoline initiated by methyl 4-nitrobenzenesulfonate.

Thermoresponsive properties were measured by UV/Vis spectrometry as dependence of transmittance at 700 nm on temperature. Thermal properties were characterized by DSC. Drug-loaded and empty nanoparticles were prepared by dialysis method and characterized by DLS. In vitro cytotoxicity of copolymers and nanoparticles were characterized by a laboratory MTT test. Cell internalization was visualized by CLSM.

Results and Discussion

2-(4-alkyloxyphenyl-2-oxazolines) representing extremely hydrophobic monomers were used in living ring-opening cationic copolymerizations with 2-ethyl-2-oxazolines resulting in the library of amphiphilic copolymers. The composition of copolymers was followed by NMR in different time of polymerization and kinetic plots proved gradient character of prepared copolymers (FIG. 1). The polarity of prepared copolymers was characterized by contact angles measurements. Copolymers with content up to 15 mol % of aromatic comonomer exhibited thermosensitive behaviour.

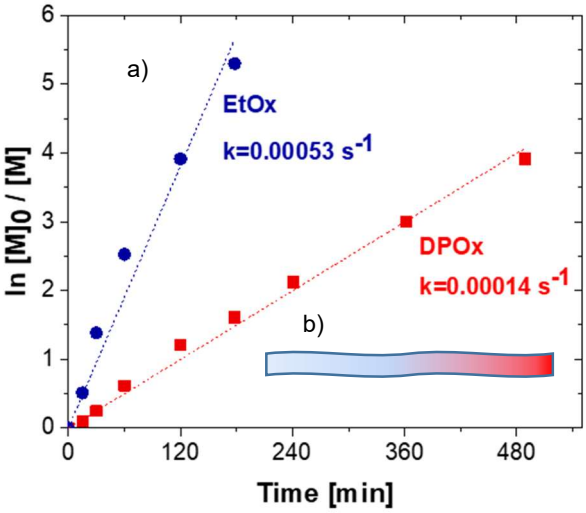


FIG. 1. Difference of polymerization rate of 2-ethyl-2-oxazoline (EtOx) and 2-(4-dodecyloxyphenyl)-2-oxazoline (DPO) during cationic polymerization (a) and schematic illustration of a gradient structure (b)

Copolymers above 12 mol.% of aromatic comonomers were able to form stable micellar nanoparticles. Particle size, shape, and loading capacity of model drugs were dependent on type of aromatic comonomers and composition of copolymers.

Conclusions

Gradient copolymers of different 2-(4-alkyloxyphenyl)-2-oxazolines with 2-ethyl-2-oxazoline represent versatile polymeric materials with thermosensitive properties and self-assembly behaviour.

Acknowledgments

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WHEY PROTEIN ISOLATE-ARAGONITE COMPOSITES FOR BONE TISSUE ENGINEERING

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[Engineering of Biomaterials 148 (2018) 97]

Introduction

Hydrogels, or highly hydrated three-dimensional polymer networks can be improved for applications in bone regeneration by mineralization to create hydrogel-inorganic composites. In this study, whey protein isolate (WPI) hydrogels were mineralized by incorporation of preformed aragonite particles. WPI is a by-product from the production of cheese and Greek yoghurt. Hence, its usage is advantageous for environmental and financial reasons. Also, previously it has been demonstrated that WPI in solution promotes proliferation and osteogenic differentiation of cells [1]. WPI hydrogels can be formed by heat sterilization, e.g. autoclaving. Aragonite is a polymorph of calcium carbonate ($CaCO_3$). It has successfully been used to promote bone regeneration [2].

Materials and Methods

Hydrogel-$CaCO_3$ composites were produced by the heat-induced gelation of 40% WPI solution, with 0, 100, 200 or 300 mg/ml aragonite particles added (denoted as WPI, WPI/100$CaCO_3$, WPI/200$CaCO_3$, WPI/300$CaCO_3$). 1 ml composites were formed in 2 ml Eppendorf tubes. Composite properties were investigated by swelling studies, degradation (BCA assay), morphology (SEM), structure (FTIR, Raman spectroscopy), mechanical properties (compressive modulus), particle distribution (Micro-CT imaging) and cytocompatibility (cell metabolic activity and alkaline phosphatase activity (ALP)) using MG63 osteoblast-like cells, after autoclaving.

Results and Discussion

Particles had a positive impact on mechanical properties. The highest compression modulus was observed in WPI/300$CaCO_3$ hydrogels *c.a* 3.15 MPa (FIG. 1). SEM and Micro-CT analyses suggested that aragonite particles were uniformly distributed within hydrogels (FIG. 2). MG63 metabolic activity and ALP activity were also highest for WPI/300$CaCO_3$ hydrogels, suggesting positive effect of aragonite incorporation on MG63 cell survival and early osteogenic differentiation, respectively.

Conclusions

Physicochemical, mechanical and cytocompatibility studies indicated that WPI/300$CaCO_3$ were most suitable for cell growth and possibly bone tissue engineering applications.

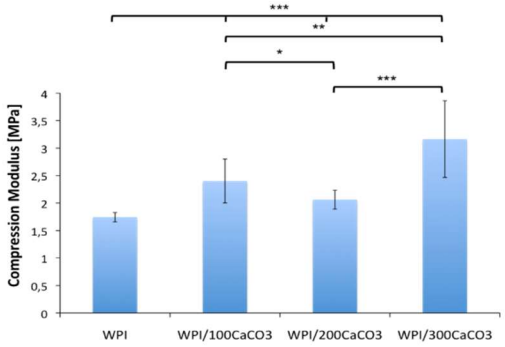

FIG. 1. Compressive modulus of composites

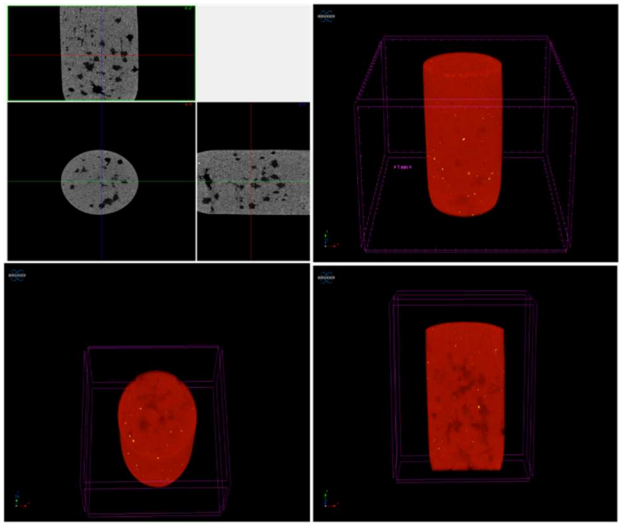

FIG. 2. Micro-CT analysis of WPI/300$CaCO_3$ composites (diameter 8 mm). Top left and bottom right: cross-sections Red: hydrogel. Yellow: $CaCO_3$

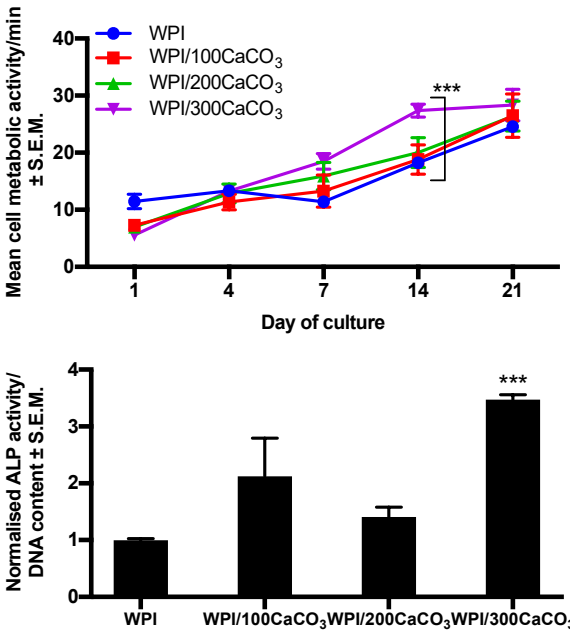

FIG. 3. Metabolic activity (top) and ALP activity (bottom) of MG63 cells on composites after 21 d.

Acknowledgment

United Kingdom Society for Biomaterials Lab-2-Lab grant, Lancaster University FST grantm, N8 Agrifood pump priming grant "Food2Bone" (all T.E.L.D.).

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DOLOMITE AND CALCITE ENHANCEMENT OF WHEY PROTEIN ISOLATE HYDROGELS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 98]

Introduction

Calcite, the thermodynamically most stable polymorph of calcium carbonate ($CaCO_3$), has successfully been used to promote bone regeneration [1]. Dolomite ($CaMg(CO_3)_2$), is a form of magnesium calcite, which is used in the building industry and is available in large amounts in Poland. Mg promotes bone-forming cell proliferation as a component of calcium phosphate (CaP) [2]. We hypothesized that Mg incorporation into $CaCO_3$ would also positively influence cell behaviour, and that addition of dolomite and calcite to hydrogels, (highly hydrated three-dimensional polymer networks) would improve cell proliferation. Addition of preformed inorganic particles to hydrogels is a common mineralization strategy [3]. In this study, dolomite and calcite particles were added to hydrogels of whey protein isolate (WPI), an inexpensive by-product from the dairy industry, and has displayed positive biological effects in our previous work [4]. WPI hydrogels can be formed by heat sterilization, e.g. autoclaving.

Materials and Methods

Synthetic calcite was prepared as described previously [5]. Dolomite was obtained from the Ołdrzychowice region of Lower Silesia, Poland, as described previously [6]. Composites were produced by the heat-induced gelation of 50% (w/v) WPI solution, with 30% (w/v) calcite or dolomite particles added (denoted hereafter as WPI-calcite and WPI-dolomite, respectively). 1 ml composites were formed in 2 ml Eppendorf tubes.

Composites were investigated by assessing particle distribution (Micro-CT imaging) and cytocompatibility. 10,000 MG63 cells were seeded on WPI-calcite and WPI-dolomite (n=5) and polystyrene (n=4). Proliferation after 1, 4 and 7 days was assessed using the fluorescent PrestoBlue assay. Fluorescence microscopy after DAPI staining was also performed.

Results and Discussion

Micro-CT analysis (Bruker) suggested good cross-sectional distribution of both calcite and dolomite particles within hydrogel-particle composites (FIG. 1).

MG63 cells proliferated on both WPI-calcite and WPI-dolomite, though to a lesser extent than on polystyrene (FIG. 2).

Cells showed a well-spread morphology (FIG. 3). Proliferation increased over 7 days. No significant differences were observed between WPI-calcite and WPI-dolomite. Further work will focus on increased physicochemical characterization of composites and cell biological characterization, possibly with primary cells instead of a cell line.

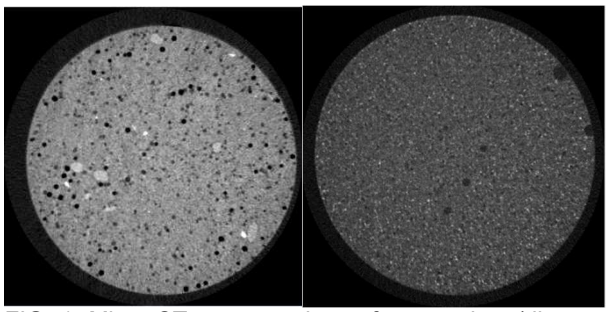

FIG. 1. Micro-CT cross-sections of composites (diameter 8 mm). Left: WPI-calcite. Right: WPI-dolomite. White dots indicate the presence of mineral. Black dots indicate cavities.

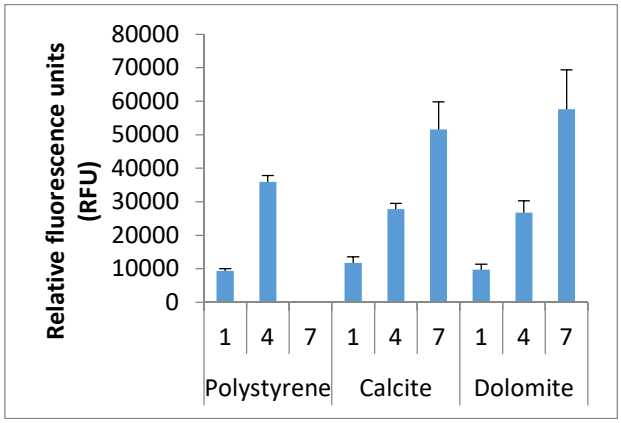

FIG. 2. Proliferation of MG63 cells over 7 days on Polystyrene, WPI-calcite and WPI-dolomite. Error bars are representative of standard deviation.

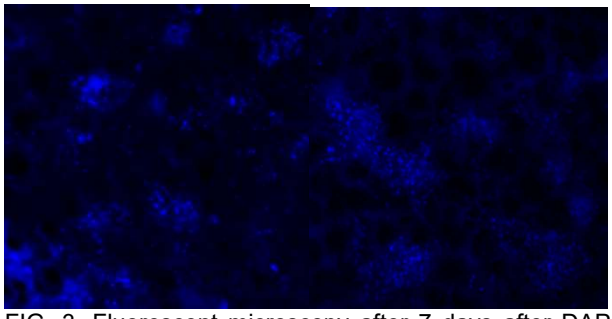

FIG. 3. Fluorescent microscopy after 7 days after DAPI staining. Magnification x5. Left: WPI-calcite. Right: WPI-dolomite.

Conclusions

WPI-calcite and WPI-dolomite composites both displayed cytocompatibility and supported MG63 cell adhesion and proliferation. Thus, both composites appear to be promising materials for bone tissue regeneration.

Acknowledgement

United Kingdom Society for Biomaterials Lab-2-Lab grant, Lancaster University FST grant, N8 Agrifood pump priming grant “Food2Bone” (all T.E.L.D.).

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THE INFLUENCE OF INCUBATION MEDIA ON SURFACE PROPERTIES OF BIOGLASS-POLYMER COMPOSITES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 99]

Introduction

Nowadays, injuries and diseases cause serious bone defects and impairments, which can lead to the urgent clinical need for reconstruction and repair of damaged bone tissue. Tissue engineering can be an effective alternative to conventional transplantation through the reconstruction of native tissue inside the body with the use of modern degradable scaffolds, stem cells and biological agents to support the process of reconstruction of damaged bone tissue. This can be achieved by using polymer-ceramic composites, which combine valuable properties of a biodegradable polymer matrix with the bioactivity of ceramic particles or fibers. In addition, the polymer matrix, due to various processing methods, creates the possibility of obtaining materials with a suitably designed microstructure. In turn, the bioactive modifying phase gives the composites unique chemical properties and allows the creation of a permanent bond with adjacent bone tissue.

The aim of this study was to evaluate *in vitro* bioactive properties of polymer-ceramic composites containing bioactive glass particles of various chemical composition designed for bone tissue regeneration, under different incubation conditions (different incubation media).

Materials and Methods

Bioglasses from SiO_2-CaO and SiO_2-CaO-P_2O_5 systems were prepared using sol-gel chemical synthesis. Then, two-dimensional composite films were fabricated with the solvent casting method and they consisted of synthetic PLGA matrix and the modifying phase composed of bioglass fillers differing in the molar CaO/SiO_2 ratio. *In vitro* bioactivity tests were carried out through immersion of the samples in several artificial fluids: simulated body fluid (SBF), cell culture medium (DMEM) and cell culture medium with the addition of fetal bovine serum (DMEM + FBS). After 7 and 14 days of soaking, samples were taken out and analyzed for the surface properties alteration, using various research methods, which included structural FTIR-ATR analysis, surface wettability and roughness determination as well as the SEM microscopy and EDS microanalysis.

Results and Discussion

Tab. 1 shows the chemical compositions of bioglasses obtained in the sol-gel process, subsequently incorporated into two-dimensional composite films.

TABLE 1. Composition of bioglasses used as modifiers in composites.

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

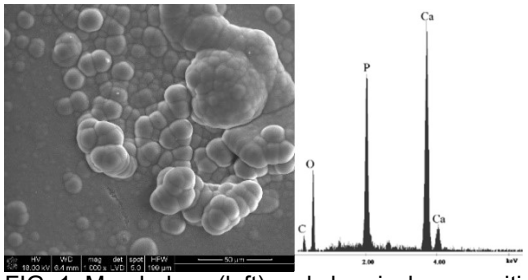

FIG. 1. Morphology (left) and chemical composition (right) of the newly formed phosphate-calcium layer on D2/PLGA composite after 14 days of immersion in SBF solution.

FTIR-ATR analysis showed that the strongest interactions occurred on samples immersed in SBF, as indicated by significant changes in the shape of FTIR-ATR spectra, while the composites incubated in DMEM and DMEM+FBS solutions exhibited lower surface reactivity. Analysis of SEM images enabled observation of layers with irregular morphology grown under the influence of incubation solutions. Additionally, by means of the microanalysis EDS, the composition of the surfaces was determined. FIG. 1 presents SEM microphotography with corresponding EDS spectrum of the newly formed phosphate-calcium layer on composites after 14 days of incubation in SBF solution. Spectra of the samples immersed in SBF showed a significant growth of phosphorus peak (P) next to the calcium peak (Ca), which indicated the formation of the phosphate-calcium layer (FIG. 1). Deposits produced on the samples in DMEM and DMEM+FBS solutions were significantly different, including needle-like morphology, lower Ca/P ratio and trace amounts of other elements (Na, Mg, Si).

Conclusions

The most preferable solution for testing the *in vitro* bioactivity of these materials, dedicated to bone tissue regeneration, was SBF. Additionally, the least bioactive materials turned out to be composites modified with the bioglasses containing the highest silica content. The addition of fetal bovine serum (FBS) to the DMEM incubation medium had an inhibitory effect on the formation of the phosphate-calcium layer on the surface of the composite materials.

Acknowledgments

This work was supported by the National Science Centre Poland Grant no. 2014/13/B/ST8/02973

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ADHESION OF BACTERIAL FILMS ON POLYMER FOOD FILMS COVERED BY GRAPHENE OXIDE EMULSION AND GRAPHENE NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 100]

Introduction

Bioactive food packaging should protect food against bacteria and fungi. An important aspect is the initiation of a protective barrier process against pathogens. Such a barrier may be a graphene oxide emulsion layer [1-3].

Materials and Methods

The study used a commercially available water emulsion of graphene oxide and three types of food films for fat products. The bacteriostatic properties of the emulsion of graphene oxide (GO) graphene nanoparticles and silver nanoparticles were investigated. The tests used standard strains that are representative pathogens: *Escherichia coli ATCC 25922, Pseudomonas aeruginosa NCTC 12903 / ATCC 27853, Staphylococcus aureus ATCC® 25923, Streptococcus mutans ATCC 35668, Streptococcus sanguis ATCC10556*.

The assessment of antibacterial activity was made using the direct method based on the criteria contained in the description of SN 195920: by modified circular diffusion according to Czerwińska.

The adhesion of bacteriological biofilms to food foils with graphene on a fluorescence microscope was then carried out (MOTIC B1410E, 400 x magnification).

Results and Discussion

The research results indicate the lack of antibacterial properties of graphene oxide emulsions (as a layer) and the strong bacteriostatic properties of graphene nanoparticles and silver nanoparticles. Fluorescence of graphene on food films was not observed in the fluorescence microscope.

Conclusions

The bioactivity of packages with graphene oxide emulsion is a protective barrier against food-borne pathogens.

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ADHESION OF BACTERIAL FILMS ON POLYMER FOOD FILMS INCORPORATED DETONATION NANODIAMOND PARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 101]

Introduction
Bioactive food packaging should protect food from bacterial and fungal pathogens. An important aspect is the attempt to integrate food packaging with detonating diamond nanoparticles in order to give it antioxidant properties [1-3].

Materials and Methods
Detonation nanodiamond particles produced by Danilenko characterized the single grain sizes from 2 to 4 nm and high chemical polarity. The bacteriostatic properties of nanodiamonds were investigated. The tests used standard strains that are representative pathogens; *Escherichia coli ATCC 25922,Pseudomonas aeruginosa NCTC 12903 / ATCC 27853,Staphylococcus aureus ATCC® 25923, Streptococcus mutans ATCC 35668, Streptococcus sanguis ATC 10556.*
The assessment of antibacterial activity was made using the direct method based on the criteria contained in the description of SN 195920: by modified circular diffusion according to Czerwińska.
Subsequently, the studies of adhesion of bacterial biofilms to food films with fluorescent nanodiamonds were carried out on a fluorescent microscope (MOTIC B1410E, 400x magnification).

Results and Discussion
The results of the study indicate the lack of bacteriostatic properties of nanodiamonds and the presence of bacterial biofilms on food films.

Conclusions
Food films with incorporated nanodiamonds do not exhibit bacteriostatic or anti-adhesive properties onto polymer food films.

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MODIFICATION OF MICROPARTICLES' MICROSTRUCTURE WITH CARBON DIOXIDE FOR APPLICATION AS CELL CARRIERS IN MODULAR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 148 (2018) 102]

Introduction

Microparticles (MP) made of resorbable polymers are considered as convenient tools to culture cells. MP may be assembled to cell constructs or suspended in hydrogels suitable for administration into the tissues by injection. High surface area of MP provides more advanced cell culture conditions than traditional culture on flat substrates. Moreover, MP with proper porosity were found to promote cell adhesion [1].
In this study we aimed to modify microstructure of MP by changing composition of both water and oil phases in the process of emulsification. Particularly we were interested in chemicals that undergo decomposition and release carbon dioxide (CO_2). Our hypothesis was that CO_2 will create pores in the MP. Moreover, we intended to find out how these modifications influence cytocompatibility of MP.

Materials and Methods

MP were prepared by oil-in-water emulsification method by pouring solution of poly(L-lactide-*co*-glycolide) (PLGA 85:15, M_n = 100 kDa, M_w = 210 kDa) in dichloromethane (DCM) to *aq.* solution of polyvinyl alcohol (PVA, Mowiol 4-88, Sigma-Aldrich, avg. M_w 31 kDa) during stirring with magnetic stirrer (250 rpm). Some of oil phases and all of water phases were modified with citric acid or Na_2CO_3. After 24 h MP were vacuum filtrated, rinsed with distilled water and left at 37°C to dry. After that MP were sieved with laboratory sieves (mesh diameter 100 µm) to receive fraction with diameters above 100 µm. Obtained MP were analysed with optical microscope (Axiovert 40, Zeiss, Keyence VHX-900F) to characterise their diameter. Additionally, to observe surface, porosity and roughness scanning electron microscopy (Nano Nova SEM 200) was used. For preliminary assessment of MP cytocompatibility MG-63 osteoblast-like cells were cultured in TCPS 48-well Nunclon plates for 1, 3 and 7 days at 37°C under 5% CO_2 in contact with 2 mg MP per well. Cell viability was measured by Alamar Blue assay (Sigma Aldrich). Live/dead (calcein AM/propidium iodide Sigma-Aldrich) and haematoxylin/eosin staining were used and the samples were observed with fluorescence and optical microscopes (Axiovert, Zeiss and Keyence VHX-900F), respectively.

Results and Discussion

Addition of citric acid and Na_2CO_3 to both phases resulted in differences of MS microstructure. MS obtained when water phase was modified were transparent (FIG. 1 a, b). When both phases were modified and Na_2CO_3-citric acid reaction took place, released CO_2 was encapsulated inside MP creating closed porosity, but also modified roughness of the MP surface. As a result MP became opaque (FIG. 1 c, d).

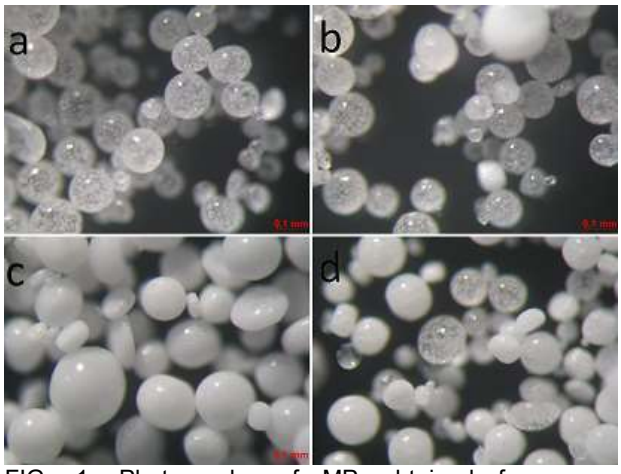

FIG. 1. Photographs of MP obtained from pure PLGA/DCM solution (a, b) poured to 1.5% PVA solution modified with citric acid (a) and Na_2CO_3 (b) and PLGA/DCM with Na_2CO_3 to *aq.* PVA with citric acid (c) and PLGA/DCM with citric acid to *aq.* PVA with Na_2CO_3 (d).

In vitro tests showed good cytocompatibility of microparticles (FIG. 2). After 7 days higher coverage with cells was observed for MP where CO_2 was produced in reaction of citric acid and Na_2CO_3 (FIG. 2 c, d) and those samples showed higher cell viability.

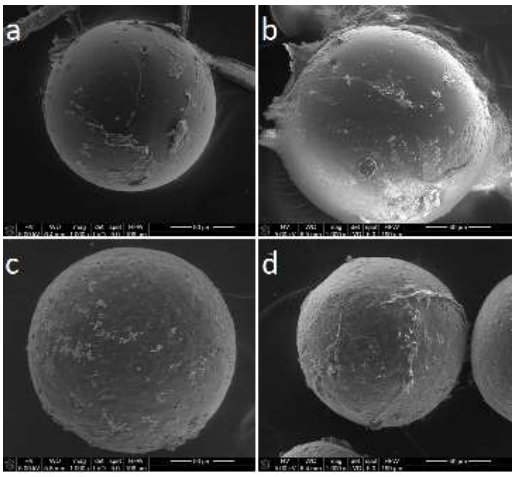

FIG. 2. SEM microphotographs of MG63 cells cultured for 7 days on MS obtained from pure PLGA/DCM solution (a, b) poured to 1.5% PVA solution modified with citric acid (a) and Na_2CO_3 (b) and PLGA/DCM with Na_2CO_3 to *aq.* PVA with citric acid (c) and PLGA/DCM with citric acid to *aq.* PVA with Na_2CO_3 (d).

Conclusions

Method of emulsification allowed to obtain MP differing in diameter, porosity and morphology. Such parameters can be easily modified by addition of other chemicals to both water and oil phases. *In vitro* tests showed good adhesion and growth of cells on MP, particularly those with modified microstructure.

Acknowledgments

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NEW TYPE OF POLYMERIC ANTYBACTERIAL COATING CONTAINING METAL NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 103]

Introduction

Nowadays epidemics are still a potential threat - the spread of bacteria through people touching various surfaces at sensitive areas (hospitals, clinics, public transport, shops, schools, etc.) Problem arises in large human agglomerations and with people migrations (in EU millions of people travel from place to place every day). Problem are also bacteria from the other continents brought with immigrants to EU countries and bacteria resistant to antibiotics. The solution to above mentioned problems is the modification of metallic (and polymer) surfaces with antibacterial coatings. In ANBACO project the different technologies of coatings deposition will be applied and as the result of the project the non-toxic antibacterial coatings with the best physico-chemical properties will be obtained. Such the coatings will find many every-day applications (in public transport, supermarkets, toilets, during travels etc.) but also will find applications in medicine [1-3].

Materials and Methods

Studies on the biocidal properties of the surface have been carried out for material coated with metal nanoparticles in accordance with ISO 22196: 2011 / JIS Z 2801: 2010 standard.
The antibacterial properties of the materials tested are determined by calculating the number of bacterial or fungal cells that survived after direct contact with the surface containing the germicide for 23 hours at 35°C. The effectiveness of the antibacterial properties of a given material is evaluated by comparing the number of surviving cells on a sample protected with an anti-bacterial agent and a control sample.
The following bacterial strains and fungal species were used in the study: Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis, Candida albicans, Candida parapsilosis.

Results and Discussion

TABLE 1.

Bacterial strain	Sample description	Time	TVC (JTK/mL) $\log_{10}$	% of reduction	Bacterial activity(*R*)
Staphylococcus aureus ATCC 6538P	Control sample	T_0	6,00		
		T_{24}	7.83		
	Test sample	T_{24}	5.15	>99	2.7
Rate:					**Good**

TABLE 2.

Bacterial strain	Sample description	Time	TVC (JTK/mL) $\log_{10}$	% of reduction	Bacterial activity(*R*)
Staphylococcus epidermidis Clinical izolate	Control sample	T_0	5,40		
		T_{24}	7,30		
	Test sample	T_{24}	5.02	>99	2.3
Rate:					**Good**

TABLE 3.

Microorganism	Sample description	Time	TVC (JTK/mL) $\log_{10}$	% of reduction	Bacterial activity(*R*)
Candida albicans Clinical Izolate	Control sample	T_0	6,0		
		T_{24}	7,08		
	Test sample	T_{24}	4.95	>99	2.1
Rate:					**Good**

TABLE 4.

Microorganism	Sample description	Time	TVC (JTK/mL) $\log_{10}$	% of reduction	Bacterial activity(*R*)
Candida albicans Clinical izolate	Control sample	T_0	6,0		
		T_{24}	7,08		
	Test sample	T_{24}	4.95	>99	2.1
Rate:					**Good**

Conclusions

The results of the conducted study indicate that the tested samples show strong antibacterial properties against Gram-positive bacteria and antimycotic properties against tested yeast isolates.

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SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES AS VERSATILE DRUG DELIVERY CARRIERS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 104]

Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) poses numerous advantages as drug delivery carriers and hyperthermia agents in cancer treatment[1]. Good magnetic properties of SPIONs allow for their precise guidance and accumulation directly at the tumour site. However unmodified SPIONs can release significant amount of iron ions. It was proved that those ions can increase proliferation of cancer cells[2]. Thus, it is crucial to modify the surface of SPIONs to reduce potential release of iron ions. Besides its protective function, surface modification of SPIONs can be utilized for attachment of anticancer drugs or other biologically active molecules. The aim of the present study was to modify Fe_3O_4 nanoparticles with different silica layers, to evaluate their physico-chemical properties and cytocompatibility with human lung epithelial cells.

Materials and Methods

Fe_3O_4 nanoparticles (NP) were coated with SiO_2 layer (non-porous: NP@SiO_2, mesoporous: NP@$mSiO_2$ or both of them: NP@SiO_2@$mSiO_2$) using a sol-gel method with TEOS as a precursor and CTAB as a progen. Chemical composition of modified SPIONs was studied by FTIR. Iron release studies were performed for 24 h in ultrahigh purity water and iron concentration was measured with AAS electrothermic method. NP size and morphology were assessed using transmission electron microscopy (TEM) and atomic force microscopy (AFM); surface zeta potential was also measured (Litesizer, Anton Paar). The hysteresis loops of the nanopaticles M(H) were measured at 5K and at room temperature with SQUID Magnetometer MPMS XL (Quantum Design). NP uptake and its influence on cell viability and migration were evaluated in vitro in contact with malignant (A549) and non-malignant (BEAS-2B) human lung epithelial cells (Prussian blue staining, real-time impedance measurement – xCelligence system). Cell migration was studied and analysed based on real-time phase contrast images (IncuCyte ZOOM).

Results and Discussion

Silica layers were successfully deposited on Fe_3O_4 nanoparticles. FTIR spectra of silica coated NP showed bands in the range of 1000-1200 cm^{-1} corresponding to Si-O-Si bonds, which were not present for unmodified NP (FIG. 1A). Surface zeta potential changed from +20.1 mV for unmodified NP to between -12.6 mV to -23.4 mV for silica coated NP. Deposition of silica layer on NP significantly decreased iron release (FIG. 1B). Formation of silica coating also influenced magnetic properties of NP. Magnetic moment of particles covered with silica was 30% smaller than for unmodified NP. This certifies that approximately 30% of modified NP is silica. We have seen the paramagnetic/superparamagnetic contribution for mesoporous and amorphous silica coating. For unmodified NP and NP@SiO_2 we have also observed the Verwey phase transition resulting from the change of Fe_3O_4 crystallographic structure from cubic to monoclinic. On the other hand, the largest coercive field was observed for NP@$mSiO_2$ and NP@SiO_2@$mSiO_2$.

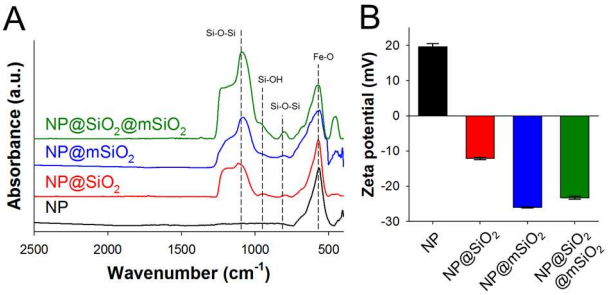

FIG. 1. FTIR spectra (A) and surface zeta potential (B) of unmodified and silica coated NP.

All types of NP were efficiently internalised by lung epithelial cells and they did not show any influence on viability of malignant lung epithelial cells at concentration of 10 µg/ml. In the case of non-malignant lung epithelial cells reduced proliferation was observed, which was correlated with more intensive cell migration and increased cell velocity as shown by real-time phase contrast analysis by IncuCyte (FIG. 2).

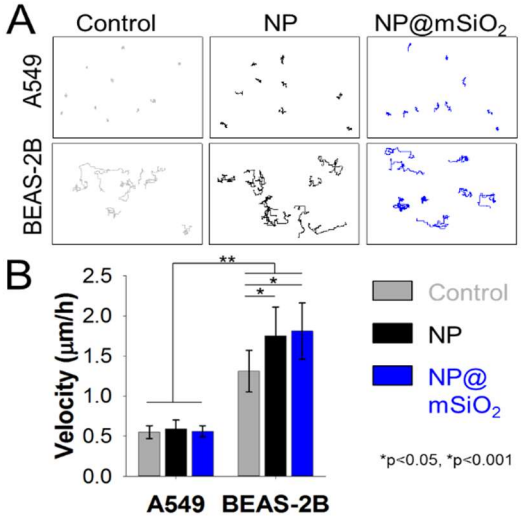

FIG. 2. Paths of single cell migration (A) and average velocity of cells (B) after addition of unmodified NP and NP@$mSiO_2$ (10 µg/ml).

Conclusions

Silica modified SPIONs are promising materials for hyperthermia and drug delivery purposes as they do not release iron ions and do not show significant cytotoxic effects towards lung epithelial cells. Silica coatings can be further modified with different drugs or biologically active molecules to enhance anticancer treatment.

Acknowledgments

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OSTEOBLAST BEHAVIOUR ON NOVEL WHEY PROTEIN ISOLATE HYDROGELS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 105]

Introduction

The first critical course for assessing the suitability of a new material in bone regeneration is the compatibility of the biosystem at the site of its effect. Knowledge of the molecular interactions material modifications with the biosystem is of specific importance.

Proteins derived from dairy sources have attracted attention for regenerative medicine. Whey protein isolate (WPI) is a by-product from the manufacture of Greek yoghurt. It contains mainly of β-lactoglobulin. Hydrogels are highly hydrated three-dimensional polymer networks which are used increasingly as materials for bone tissue regeneration. WPI hydrogels are formed by heating WPI solution, a well-known phenomenon in the food industry.

Osteoblasts are the important cell type in orthopedic applications. The most important initial process in this cell-material interaction is the mechanical anchoring of the cell to the material interface – the cellular adhesion (FIG. 1) [1]. External signals from physico-chemical environments finally affect the cell physiology [2]. Therefore, it is important to study *in vitro* effects of hydrogel composition on cellular adhesion, and cell growth, organization of the actin cytoskeleton, and proliferation [2].

Previous work has shown that WPI improves cell proliferation and osteogenic differentiation [3]. The application of WPI hydrogels in tissue engineering has still not been explored. The understanding and interpretation of the cellular behaviour is critical for the acceptance of novel materials on bone regeneration.

Materials and Methods

WPI hydrogels were formed by autoclaving WPI solution, i.e. by performing fabrication and sterilization in one step. Various WPI concentrations (20, 30, 40, 50% all w/v) were compared and subjected to compressive testing. Young's modulus increased with rising WPI concentration (FIG. 2). The first *in vitro* studies, to conduct the acceptance of novel whey protein isolated hydrogels, were performed with osteoblasts (MG-63, ATCC® CRL-1427™). The cells were cultured within 24h in DMEM with 10% FCS (Biochrom) under physiological conditions: 37°C, 5 % CO_2 [2]. To analyse the cell adhesion and growth we used microscopy – scanning electron microscopy (FE-SEM), confocal laser scanning microscopy (LSM), and flow cytometry. To validate the data we used GraphPad Prism with the corresponding test of significance.

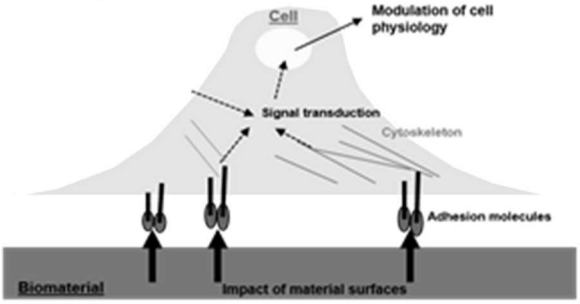

FIG. 1. Scheme of cell-material interaction.

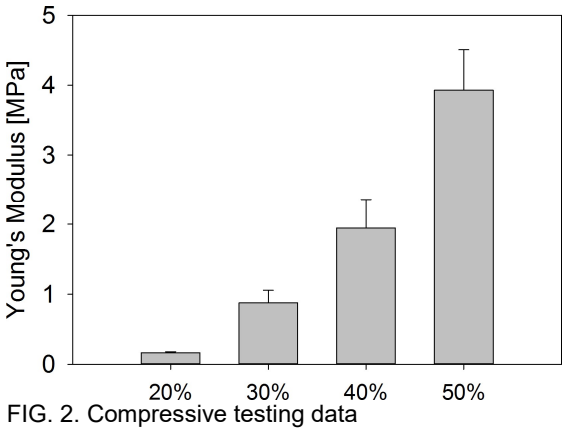

FIG. 2. Compressive testing data

Results and Discussion

Established on our *in vitro* studies, we were able to demonstrate that changing the concentration of whey protein hydrogels influences the osteoblast behavior: changes in cell morphology, the organization of cellular structures, and finally proliferation. Morphological analyses with FE-SEM revealed the cellular behaviour on the hydrogels; this is an important parameter. Using LSM we were able to recognize the influence of hydrogels on the spatial organization of cellular components, e.g. the actin cytoskeleton. Cell growth and spreading occurred on hydrogels, particularly on 40% and 50% hydrogels compared to 20% and 30% hydrogels. These studies of the cell physiology and cell adhesion are first important steps for assessing cellular behavior at the interface of a material with a biological environment.

Conclusions

WPI hydrogels show potential as biomaterials for bone tissue engineering. Further work will focus on osteogenic differentiation studies. Cell biologic *in vitro* studies are necessary for a better understanding and assessment of innovative medical materials and their interplay with the surrounding biosystem. A material for bone tissue engineering should support attachment and proliferation of bone-forming cells.

Acknowledgments

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NEW APPLICATIONS OF FeMOF AS DRUG DELIVERY SYSTEM FOR THEOPHYLLINE

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[ENGINEERING OF BIOMATERIALS 148 (2018) 106]

Introduction

Over the past few years, the rapid development of pharmaceutical science has led to the creation of multiple drug delivery platforms. However, search continues for more versatile solutions that will be able to handle many different routes of administration and fulfil additional applications, e.g. as theranostics. One of the promising drug carriers are MOF materials (Metal-Organic-Framework). It is a group of porous solids built of metallic clusters and organic connections between them, so-called linkers. The formation of coordination bonds between building blocks allows obtaining three-dimensional structures with well-developed surface and extremely large internal space. As a result, MOFs are able to encapsulate drugs and its controlled release [1]. Additionally, G. Wyszogrodzka and coworkers proved that MOF materials could be used in inhalation treatment of tuberculosis [2]. On the basis of the studies, FeMIL-100 MOF material was chosen as a matrix suitable for the inhalation route of administration. The aim of this study was initial evaluation of the properties of FeMIL-100 as a drug carrier of theophylline in terms of its functionality, i.e. drug loading, kinetics of release and biological effect on epithelial human cells.

Materials and Methods

Synthesis and characterization

FeMIL-100 was synthesized according to the procedure described by Guesh et al [3]. The obtained material was examined by PXRD and FTIR techniques.

Preparation of composite

In the first step, 300 mg of Fe-MIL-100 was activated under vacuum at 110°C. After 6 hours of activation, the saturated solution of theophylline in what solution was injected into the glass vial and mixed together with MOF material for 24 hours. After that, the solvent was removed by what treatment. Obtained composite was washed three times with distilled water and dried at room temperature overnight.

Drug release

The drug release was carried out in Franz cells. Gamble's solution (pH=7,4) was used as the medium, simulating lung environment. 5 mg of the composite was placed in the membrane. After 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 24 and 48 hours, an aliquot of 0,1 ml was withdrawn and replaced with the same volume of fresh dissolution medium.

Cell study

Epithelial human cells (A549, ATTC, USA) were cultured in F-12 medium (ATTC, USA) supplemented with 10% foetal bovine serum (ATTC, USA). Murine macrophages (RAW, ATTC, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma Aldrich, USA). The cells were cultured in optimal condition at 37° C, 5% CO_2, and 95% humidity. After passage 3rd, cells were seeded at a density of 5 x 10^3 cells per well (200 µl/well), kept under culture conditions and allowed to adherent. The biocompatibility of the MOF was analysed after 1 and 3 days of the culture with the use of PrestoBlue™ assay (Invitrogen, USA). The level of ROS generation was determined using DCF-DA probe. Depending on the assay, the intensity of the signal of fluorescence or luminescence was measured on the microplate reader POLARstar Omega (BMG Labtech, Germany). Cells morphology was controlled using optical microscope. For all tests, three independent repetitions of each measurement were performed. All data are given as mean ± standard error of mean (SEM).

Results and Discussion

In this study, FeMIL-100 was chosen as a new potential drug carrier for theophylline. PXRD and IR measurements confirmed that drug was placed inside the structure [FIG. 1]. The drug dissolution studies showed the extended release of theophylline. Based on in vitro study, FeMIL-100 is not toxic for epithelial human cells [FIG. 2]. The ROS level was elevated after 1-day incubation but after 3 days it returned to the value characteristic of the control group [FIG. 2]. This effect may be connected with shock for cells after water solution of MOF was added but it did not influence the viability of cells.

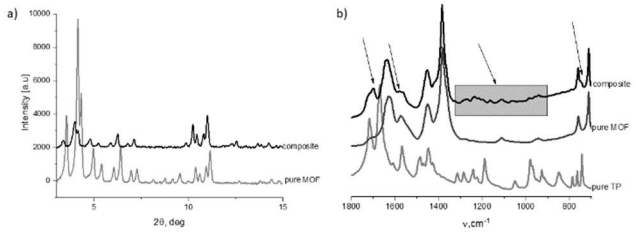

FIG. 1. a) PXRD patterns of FeMIL-100 after and before encapsulation; b) IR spectra in framework vibration region of pure theophylline (TP), pure MOF and composite.

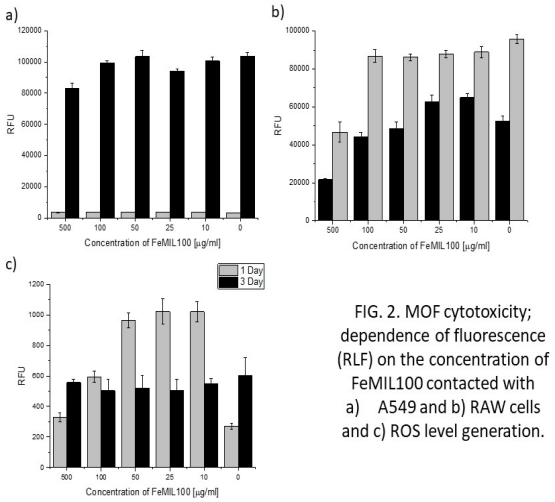

FIG. 2. MOF cytotoxicity; dependence of fluorescence (RLF) on the concentration of FeMIL100 contacted with a) A549 and b) RAW cells and c) ROS level generation.

Conclusions

Based on data gathered from the viability test it is proven that the FeMIL-100 is not cytotoxic and is not negatively influencing the growth of cells. Moreover, it is a potential carrier for theophylline and it is suitable for potential inhalation treatment.

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MOLECULAR CORRELATES OF JOINT PAIN INTENSITY IN OSTEOARTHRITIS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 107]

Introduction
Chronic pain is a main symptom of osteoarthritis (OA). Patients living with OA often exhibit abnormal movement patterns primarily due to altered joint kinematics. While some of these gait changes in OA are due to deterioration in joint congruency, compensatory movement to minimize joint loading and pain is also likely to play a part. Monitoring animal movement following arthritis induction could reveal some interesting insights into pain perception. While OA is perceived as a structural disease, the underlying pathology and chronic changes occur also at a molecular level. The gene expression alterations associated with OA are important in order to understand its initiation and progression.

Materials and Methods
Intrarticular (i.a.) injection of monosodium iodoacetate (MIA, 1mg) has been used to induce OA in Wistar rats. Pain symptoms were assessed by behavioral tests: knee joint hypersensitivity test, tactile allodynia test and kinetic weight bearing (KWB), a novel instrument designed to measure time and spatial distribution of a weight bearing by each paw of a freely moving rodent. We also applied whole-transcriptome profiling study (Affymetrix GeneChip Rat Gene 2.0 ST) in order to compare data between healthy and various OA states to better understand the mechanisms underlying a disease.

Results and Discussion
KWB is a novel reliable method for pain perception studies. It allows quantification of various locomotor parameters in spontaneously moving animals. The dynamic profiles of transcriptional changes were assigned to cellular compartments of the knee joint. The presented study identified groups of co-regulated genes that share functional relationships and may play an important role in the early and intermediate stages of OA.

Conclusions
Complex description of locomotor activity of rats in MIA model of OA allows improving accurateness in rating in OA-associated pain. This might be of benefit in assessing effectiveness of novel treatments and better transitioning results from laboratory conditions to clinic
Our study provides evidence that the progression of cartilage damage is driven by complex but precise regulation of gene patterns that are induced or suppressed during various stages of cartilage damage. The results also suggest that the observed molecular alterations are located in the specific cellular compartments of the knee cartilage. The presented classification of transcriptional alterations associated with the development of cartilage degeneration provides guide the development of new therapeutic strategies.

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PREPARATION AND CHARACTERIZATION OF GLASS-CERAMIC MATERIALS MODIFIED WITH IRON OXIDE WITH ADDITION OF SrO AND ZnO

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[ENGINEERING OF BIOMATERIALS 148 (2018) 108]

Introduction

Magnetic materials represent advanced solutions suitable for a wide range of biomedical applications, according to their different magnetic responses to an applied external magnetic field [1]. Bioactive glasses are known to be osteoinductive materials as their ionic dissolution products stimulate the cell genes toward a path of regeneration and self-repair [2]. Biocompatible magnetic glasses have attracted the attention for possible use in the advanced treatment of bone cancer as a complementary approach to chemotherapy, which is known to carry many side effects to patients. Fe_3O_4-containing melt-derived glass-ceramics were found highly promising for the treatment of osseous tumors by hyperthermia [3, 4]. Glass modification with ZnO and SrO can bring two benefits. On one hand, Zn and Sr ions released from glass structure are known to support osteoblast function and bone regeneration [5]. On the other hand, the presence of these both oxides can lead to the formation of magnetic phases after glass crystallisation, i.a. zinc ferrite and strontium ferrite [6,7].

Materials and Methods

The chemical composition of the bioglass applied in the experiment was SiO_2 – CaO – P_2O_5 – Na_2O with Fe_2O_3 modifiers and with ZnO and SrO addition. Glass-ceramic materials were produced by melting, followed by a process of directed crystallization. Four types of samples with various amounts of iron oxide and additions of zinc oxide and strontium was obtained.

The produced materials were analyzed from thermal (thermogravimetry, differential thermal analysis and differential thermal calorimetry) and microstructural (X-ray diffraction) viewpoints. SEM and EDS were applied to present the results of the study.

Results and Discussion

Differential thermal calorimetry (DSC) were performed to determine the thermal parameters of the sample: the glass temperature (Tg) and the crystallization temperature (Tc). The Tthermogravimetry (TG) analysis was performed for selected materials before and after the controlled crystallization.

For phase and structural analysis, X-ray diffraction for samples before and after the crystallization process was execute. The investigation on glass samples before the directed crystallization process confirmed their amorphous structure. The obtained results indicate that in the materials a ferritic phase with a hexagonal structure and structure with inverted spinel present.

Scanning electron microscopy (SEM) and EDS techniques were performed on the glasses before and after soaking in SBF solution. The samples were removed from this solution, washed with distilled water and dried at room temperature. SEM and EDS measurements indicated the presence of apatite layer formed on the surface of the prepared glass ceramics after immersion in SBF within 7 to 21 days. The investigation of the results clarified that the addition of iron oxide causes the formation of apatite on the surfaces of the samples in the simulated body fluid.

Conclusions

Using the method of melting and directed crystallization of bioglass modified with iron oxides with the addition of zinc oxide and strontium oxide, is possible to obtain glass-ceramic materials containing zinc and strontium ferrite. X-ray diffraction confirmed presence of crystallite phase from ferric. SEM analyze confirmed the bioactive properties of the materials obtained.

Acknowledgments

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IMPACT OF CARBON NANOFORMS ON HIPSC-DERIVED CARDIOMYOCYTES

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ENGINEERING OF BIOMATERIALS 148 (2018) 109]

Introduction

Human induced pluripotent stem cells (hiPSCs), generated from somatic cells after overexpression of defined transcription factors (most often OCT4, SOX2, KLF4 and c-MYC) demonstrate capacity to efficiently differentiate into cardiomyocytes (hiPSCs-CM). Due to such properties, hiPSCs provided novel opportunities: i) to obtain patient-specific cardiomyocytes ii) to utilize such cells in investigating mechanisms of heart diseases and in drug testing, iii) to generate cells applicable in regenerative medicine [1]. One of the key challenges in the successful usage of hiPSCs-CM in medicine is guaranteeing their proper growth, proliferation and maturation. As current state of the knowledge regarding behaviour of stem cells and their progenies within the body suggests, this cannot be done without mimicking interactions that are naturally occurring between the cells and the extracellular matrix (ECM) [2]. Those interactions could possibly be mimicked by using carefully selected scaffolds. Materials of different morphology, stiffness, chemical composition and electrical conductivity, should be able to dictate the stem cells' fate, both *in vitro* and *in vivo* [3-5].

Due to chemical and morphological biomimetism, good biocompatibility and excellent electrical conductivity, nanoforms of carbon are currently regarded as promising materials in fabricating new generation of scaffolds for culturing various cell types [6-8]. In this preliminary study, an impact of the morphology and chemical composition of two nanoforms of carbon (NC, namely, carbon nanotubes and graphene) on the viability, proliferation and maturation of the hiPSCs-CM was evaluated. Additionally, ability of NCs to activate heme oxygenase-1 (HO-1), a major cytoprotective factor demonstrating anti-inflammatory, anti-apoptotic and pro-angiogenic properties which also regulates cardiac differentiation of pluripotent stem cells was analysed [9]. Two forms of contact were tested – NCs used as matrix additive in Geltrex ® and in the form of layers deposited on the surface of titanium. The aim was to investigate their potential applicability as scaffolds that are to be electrically stimulated in order to enhance cardiac differentiation of hiPSCs and maturation of hiPSCs-CM.

Materials and Methods

Oxidized CNTs were fabricated according to the procedure established in our previous studies [10, 11] while oxidized graphene oxide (GO) was purchased from the NanoAmor Inc. and used as-received. Both NCs were subjected to additional treatment involving modification with electrically conductive polyaniline (PANI, emeraldine salt, Sigma Aldrich) to introduce NH group into the material. Chemical composition and morphology of the NCs were tested via XPS, FTIR, SEM and TEM.

For the obtainment of the layers, electrophoretic deposition was performed on the surface of degreased, and etched in 5% HF titanium. Modification of Geltrex ® was done by mixing 2 ml of NC/DMEM/F12 suspension (0.05mg/ml for CNTs and 0.1mg/ml for GO) with 20 µl of Geltrex ®. 250 µl of each of the the as prepared mixture were then pippeted into 8 separate culture wells. Geltrex ® in DMEM/F12 were used as a reference. 4,5 x 10^4 hiPSCs were seeded on Geltrex ® (control) and CNT-modifed Geltrex ® and upon reaching confluency, cardiac differentiation was initiated according to previously established protocol [12]. Briefly, on day 0 cells were stimulated with CHIR99021 (WNT pathway activator) in RMPI medium supplement with B27 lacking insulin to induce transition into mesoderm and subsequently stimulated with IWR-1 (WNT pathway inhibitor) on day 3 to form cardiac mesoderm. Further process was performed in RMPI medium supplemented with B27. On day 20, generated cells were collected and the efficiency of differentiation was analysed using FACS-based quantification of troponin T positive cells. Additionally, 5 x 10^4 hiPSCs-CM were seeded on Geltrex ® (control) or different NC-based scaffolds, collected 24h, 48h and 72h after seeding and counted to evaluate the effect of NCs on attachment and proliferation of cells.

Results and Discussion

- Efficiency of differentiation is not impaired when the cells are cultured on the NCs-modified Geltrex ®
- When in the form of a layer, NCs were found to inhibit the cells adhesion, with stronger effect observed for the PANI – modified materials. At the same time, in all of the materials there is a trend of enhancing the cells proliferation - a stable, linear growth in the cell number is observed throughout the experiment
- There is an observable tendency of NCs not stimulating the cells' maturation and increasing the expression of the HO-1 in the tested cells, possibly by inducing the oxidative stress.

Conclusions

In this study, biofunctionality of NCs as potential scaffolds for culturing the hiPSCs-CM was evaluated. The materials were found to be non-cytotoxic. When used as Geltrex ® matrix additive, the NCs did not impair the efficiency of the cells differentiation, while in the form of the layers, the NCs were found to induce higher expression of HO-1 in cells which may be beneficial in protecting against potential pathologies developing in un-matured culture cells.

All in all, the NCs were found to be interesting materials for future cultures involving electrical stimuli to enhance the cells' differentiation and maturation.

Acknowledgments

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POLY LACTIC-*CO*-GLYCOLIC ACID (PLGA) AS BIODEGRADABLE LAYER USED IN CARDIOLOGY

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Introduction

Biocompatible PLGA can be found amongst a few synthetic polymers approved for clinical trials. As a biodegradable material, PLGA is totally reabsorbed in the organism within, depending on the given literature, from three weeks to several months. It is a material with a relatively short degradation time when compared to homopolymers PLA or PGA. Literature also differentiates the content of the respective components of polymers as a factor that modifies final properties of the composite [1]. Due to inherent limitations of co-polymer PLGA resulting from its construction, its various modifications aiming at increasing or decreasing its elasticity or degradation time depending on application are under way. It was found that PLGA, as a material featuring high biocompatibility and optimal degradation time, may also serve as a matrix for medicine or genes application, both independent and in connection with metallic implants [2,3].

Materials and Methods

The aim of the study was to analyze the effect of modifying the surface of metallic biomaterials (AISI 316LVM, L605, cpTi(Grade)) by applying layers of biodegradable poly(d,l – lactide – co - glycolide) 85:15 10% on the electrochemical properties. The surface was modified by the imposition of a single layer of PLGA by dip coating sol-gel method.

Potentiodynamic studies were performed using a potentiostat PGP-201 Radiometer Analytical SAS. As reference electrode a saturated calomel electrode NEK KP-113 was used, and as the auxiliary electrode - platinum PTP-201. To determine the values characterizing the corrosion resistance of tested samples, Stern method was applied. In order to assess the integrity of imposed PLGA layer ion permeability test was carried out after different times of exposure in the artificial plasma. The concentration of ions which permeated the solution was measured using a spectrometer JY 2000 manual Yobin-Yvon.

Measurements were carried out both on samples coated with PLGA and subjected electrochemical polishing process, before and after the 36-day and 72-day incubation in a solution of artificial plasma (pH 7.0 ± 0.2) at T = 37 ± 1°C.

Results and Discussion

Application of the layer of 10% poly(lactic-*co*-glycolic acid) 85:15 onto the surface of AISI 316LVM, L605 and cpTi (Grade4) results in substantial increase of resistance to pitting corrosion of those materials in the environment of artificial plasma simulating human blood. In all cases, a favourable increase of corrosion potential E_{kor} and polarisation resistance Rp was detected, and for steel Cr-Ni-Mo and cobalt alloy also the increase of the area of perfect passivation.

TABLE 1. Results of potentiodynamic test.

Sample	E_{corr}, mV	E_{tr}, mV	Rp, kΩcm^2
0 days exposure in artificial plasma			
316LVM	-166	+1050	293
316LVM (PLGA)	-111	+3100	1320
L605	-326	+870	947
L605 (PLGA)	-270	+1870	4250
cpTi	-235	>+4000	460
cpTi (PLGA)	-182	>+4000	7340
72 days exposure in artificial plasma			
316LVM	-187	+820	114
316LVM (PLGA)	-127	+770	83
L605	-274	+850	289
L605 (PLGA)	-132	+640	255
cpTi	-215	>+4000	393
cpTi (PLGA)	-173	>+4000	253

Ion permeation tests confirmed that the suggested layer of biodegradable polymer PLGA creates a sufficient protective barrier against the impact of artificial plasma, causing at the same time a significant decrease of the number of metallic ions that penetrated the solution, in comparison to the samples that were not covered, which was shown during 72-day exposure to artificial plasma at the temperature T = 37 ± 1°C - tab. 2.

TABLE 2. Density of the mass of ions of metals permeating the solution as the result of 72-day exposure to artificial plasma.

Sample	Elements						
	Fe	Cr	Ni	Mo	Co	W	Ti
	μg/cm^2						
316LVM	0,600	0,333	0,095	0,021	-	-	-
316LVM (PLGA)	0,276	0,138	-	-	-	-	-
L605	-	0,025	0,091	-	1,833	3,002	-
L605 (PLGA)	-	-	-	-	0,321	0,536	-
cpTi	-	-	-	-	-	-	0,933
cpTi (PLGA)	-	-	-	-	-	-	0,362

Conclusions

Based on these results, it was found that the imposition of 85:15 PLGA (10%) layer on the surface of metallic biomaterials improves resistance to pitting corrosion in human blood environment. It was also found that the applied layer non constitutes an adhesive environment for plasma chemical compounds, without exposing the metallic substrate. The proposed PLGA polymer layer effectively reduces the amount of metal ions which leaked into the solution during exposure in the artificial plasma. In conclusion, the study clearly shows that modification of the implants for contact with blood surface by applying a coating of a biodegradable polymer PLGA has a positive effect on their physicochemical and electrochemical properties, thus allowing the safe use in the environment of human blood.

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SURFACE MODIFICATION OF METALIC BIOMATERIALS WITH ANTIBACTERIAL COATINGS

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Introduction

The first phenomenon that occurs after the introduction of the biomaterial into the biological environment is the creation of a biofilm on its surface. Biofilm is a form of aggregation of bacteria, fungi and other microscopic organisms in the form of thin deposits forming on various surfaces, contacting, for example, with body fluids. The natural bacterial flora of the patient is responsible for the formation of the biofilm. The presence biofilm can lead to the disappearance of the surrounding osseous tissue and, as a result, disturb the osseointegration process [1] Currently, prevention of bacterial infections is carried out using antibiotic therapy, however due to many problems associated with the way of administering the drug and its effective action, new method of administering the drug to the patient are still being sought. In order to limit those negative consequences, the physicochemical properties of the surface layer of implants are formed. As of today, different approaches are used to apply new biomaterial modification techniques [2]. So far, no satisfying study results were provided in this area of expertise. Numerous publications in the world literature (mainly in medical journals) confirm this activity. However, they most often present the most partial results of the research, which do not allow to fully assess the suitability of the produced coatings. In many papers, also the role of surface processing of metallic biomaterial is not emphasized. Therefore, the primary objective of the study is to observe the impact of physicochemical properties of the surface layers (bactericidal) on the processes occurring on the implants surface made of metallic biomaterials used in bone system.

Materials and Methods

The study material was Ti-6Al-4V alloy in the form of disks of the following dimensions: diameter, d = 14 mm, thickness, g = 2 mm. The samples were subjected to metal finishing consisting of grinding and mechanical polishing and then coating with layer of TiN by means of PVD methods. Analysing the mechanical properties, adhesion of applied layers to the metallic base was examined. What is more, during physicochemical properties evaluation, the testing of surface wettability and corrosion resistance by means of potentiodynamic method was performed

Adhesion was tested using the scratch test, in accordance with the standard [3]. In order to determine the surface wettability of the selected samples, the wetting angle and surface free energy (SFE) were evaluated with the use of Owens-Wendt method. The wettability angle measurements were performed with two liquids: distilled water (θw) (by Poch S.A.) and diiodomethane (by Merck). Measurements with a drop of liquid and diiodomethane spread over the sample surface were carried out at room temperature (T = 23°C) at the test stand incorporating SURFTENS UNIVERSAL goniometer by OEG and a PC with Surftens 4.5 software to assess the recorded drop image.

The potentiodynamic tests were carried out as recommended by the ASTM F2129 standard [4]. The test set up consisted of the VoltaLab PGP201 potentiostat, the reference electrode (type KP-113 saturated calomel electrode SCE), the auxiliary electrode (platinum wire), the working electrode (test sample) and a PC with VoltaMaster 4 software. Evaluation of the pitting corrosion was carried out in the environment of Ringer solutions with the chemical composition recommended by the standard at the temperature T = 37±1°C, and pH = 7.0±0.2

Results and Discussion

The first stage involved the assessment of electrochemical properties, under which potentiodynamic studies were carried out. Based on the obtained results, it was found favourable influence of mechanical polishing on corrosion resistance of Ti-6Al-4V titanium alloy without surface layer. Furthermore, an adverse effect of the applied TiN layer on the corrosion resistance was observed irrespective of the method of surface preparation. The conducted surface wettability tests for the samples in the initial state and with the applied titanium nitride coating showed that all samples are hydrophilic in relation to the contact angle values obtained, falling in the range below 90°. In addition, it was also found that the type of surface modification has a slight impact on its wettability, due to the similar values of the contact angle both for samples without a layer and with a TiN layer. The last study was to assess the adhesion of layers to the substrate by scratch test. On the basis of the obtained results, the influence of the method of preparing the substrate on the adhesion of the applied TiN layer was found. For a previously ground specimen, a higher critical force value was noted, causing delamination of the layer compared to a polished and grinding polished sample. During the test there was no acoustic emission signal, which indicates that the binding energy between the coating and the substrate was too low. The obtained results, however, indicate the need for further testing of the surface layers thus produced using PVD to determine their suitability for medical devices.

Conclusions

The results may provide basis to develop more specific criteria for assessing the final quality of medical products used in osseous system, which will provide the required biocompatibility of implants and contribute to minimize the risk of postoperative complications. The obtained results will make a significant contribution to the explanation of processes occurring on implants surface using in osseous system.

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SYNTHESIS AND CHARACTERIZATION OF POLYURETHANE-BASED BIOMATERIALS FOR ORTHOPAEDIC APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 112]

Introduction

Polyurethanes (PUs) are important biopolymers in clinical practice. PUs were introduced as potential biomaterials in the late 1990's. Since then polyurethanes were applied as bone cements, scaffolds or drug delivery systems. They are (co)polymers containing soft and hard segments. The soft segments are composed of polyester, polyether or polycarbonate diols, while the hard segments are formed by reaction between a chain extender and a diisocyanate. By varying the symmetry and chemical structure of the diisocyanate, the molecular weight and type of the polyol, the soft/hard segment ratio, the polymerization method and the crystallisation ability of the soft and hard segments, the physical and mechanical properties can be tuned to the target values for specific biomedical applications. Because many factors need to be considered to achieve the perfect properties for a specific biomedical application, morphology, structure, mechanical and thermal properties relationships are of crucial importance. In this context, the main goal of the research is to manufacture and characterize bioactive, biodegradable polymeric bone cements based on non-toxic PUs and polysaccharides [1-4].

Materials and Methods

Polyurethane-based materials were obtained in a two-step as well as one-step bulk polymerization method using poly(ethylene glycol) (PEG) with average molar mass 2000 and 1,6-hexamethylene diisocyanate (HDI). PUs have been synthesized with BDO (1,4-butanediol) and starch as a chain extender. All reagents were supplied from Sigma-Aldrich and were used as received without further purification. PEG was applied to absorb the exothermic heat of the PU polymerization reaction and thus to prevent a significant increase of temperature within the cement system. Before starting the synthesis of bone cement, PEG was dried under vacuum at the temperature of 110°C for 2 h. Samples were prepared with both BDO and starch and without BDO or starch. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), thermogravimetry (TG) techniques and tests of mechanical properties were used for characterization of the obtained PUs.

Results and discussion

In the first stage, characterization of all used raw materials was performed, followed by elaboration of a methods for obtaining polymer bone cements. The structure of the received PU was confirmed by FTIR technique – FIG. 1. The characteristic absorption band at 1102–1095 cm^{-1} is caused by asymmetric stretching vibrations of -C-O-C-. The band located in the range 3333-3319 cm^{-1} proves presence of NH stretching vibrations. Band at 2929-2922 cm^{-1} and 2861- 2858 cm^{-1} was assigned respectively to asymmetric and symmetric vibrations of CH_2 group. As it can be seen (FIG. 1) there is no significant shift in location of vibration bands in the samples modified with BDO and starch. Results of measurements of heat of phase transitions and temperature of melting by DSC method are presented in FIG. 2. One can see from FIG. 2 that the highest heat of phase transition was observed for the sample PU_1:BDO.

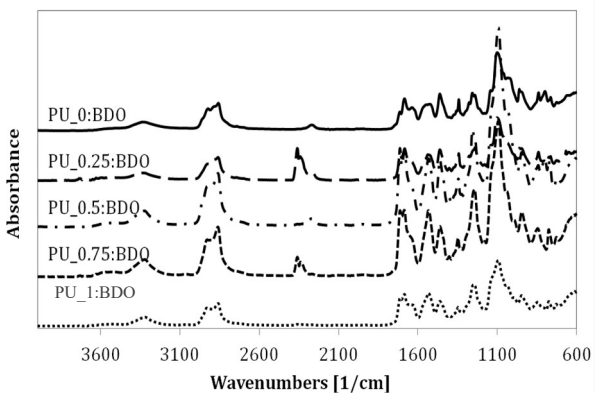

FIG. 1. FT-IR spectra of PUs samples (one-step bulk polymerization method).

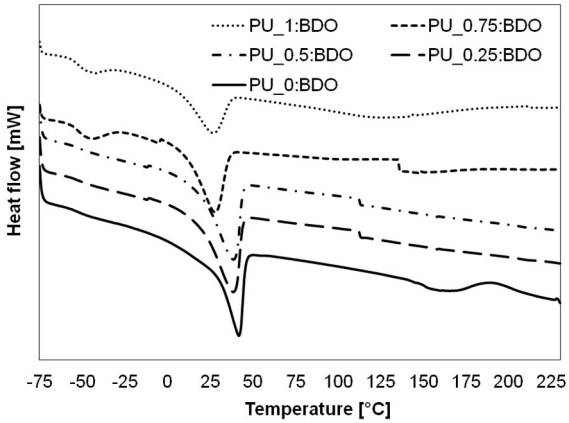

FIG. 2. DSC profiles of PUs samples (one-step bulk polymerization method).

Conclusions

Polyurethane-based materials modified with starch and BDO were obtained by applying one- or two-step bulk polyaddition method. The structure of the obtained PUs was confirmed by FTIR technique. An increase of starch content in PU materials cause a drop of heat of phase transition and an increase in the melting temperature. The obtained materials have a high potential for their further use in medicine as multifunctional bone cements.

Acknowledgments

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

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MODIFICATION OF POLY(LACTIC ACID) (PLA) AND POLY(LACTIDE-CO-GLYCOLIDE) (PLGA) FIBRES BY CERAMIC PARTICLES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 113]

Introduction

Fibres are a very interesting research object. Commercially available PLA fibres are obtained by melt spinning [1,2]. PLA and other aliphatic biodegradable polyesters have wide range of advantages - degradation ability (in body and in natural environment conditions), thermoplastic character, polymer solubility and processability by various techniques, making this group of polymers so attractive in medical and technical applications [1,3-5].
The objective of the study was determination of the influence of nano hydroxyapatite (HAp) and nano tricalcium phosphate (β-TCP) on the structure, properties and degradation behaviour of poly(L-lactide-co-D,L-lactide) (PLDLA) and poly(L-lactide-co-glycolide) (PLGA) fibres formed by the wet spinning process.

Materials and Methods

Fibres were made with Resomer LR (PLDLA - 70:30) and LG (PLGA - 82:18), medical grade product from Evonik (Germany). Two ceramic nano additives were used: HAp and β-TCP, a commercial product from Sigma-Aldrich.
Fibres were formed by wet spinning from 18.5% solutions of the PLDLA and PLGA in methylene chloride.
The fibres were drawn in several steps at increasing temperatures. The exact parameters of the coagulation and drawing process are protected by the Polish patent PL 399819 (2014) [6].
Fibres degradation studies were carried out in a simulated *in vitro* conditions at 37°C, in phosphate-buffered saline (PBS) and Ringer solution during 20 weeks.
Experimental part consisted of investigation performed on the fibres before, during and after degradation:

- intrinsic viscosity (Ubbelohde viscometer)
- tensile strength (Instron according to PN-EN ISO 5079:1999)
- microscopic structure (scanning electron microscopic SEM JSM 5400 J and SEM+EDS X-ray microanalyst FEI NOVA nanoSEM 23).

Results and Discussion

Research focused on determination the influence of basic parameters of the forming process on the structure and properties of PLDLA and PLGA fibres modified with ceramic nano particles enabled to obtain fibres with tenacity appropriate for further processing and medical use [7,8] (protected by the Polish patent PL [6]). Tensile strength in the range 140 - 300 MPa allows to use the fibres as a component of a polymer-fibrous composite supporting the bone tissue regeneration process. Ceramic particles introduction into a fibrous structure impacted into a different behavior of both polymers during wet spinning process, which end effect can be observed by tenacity decrease (FIG. 1). During degradation process an influence of ceramic particles has been observed (FIG. 2). Progressive degradation of the fibres material has been noted (TABLE 1).

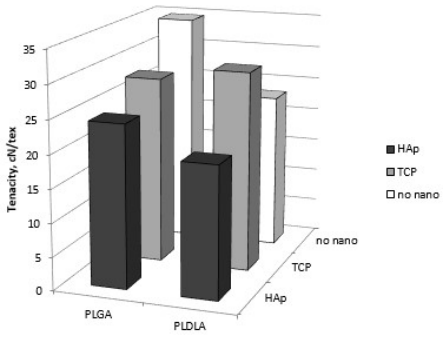

FIG. 1. Tensile strength properties for PLDLA and PLGA fibres.

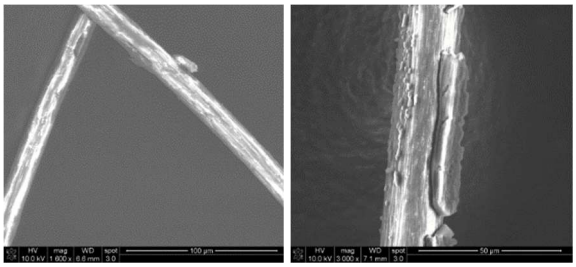

FIG. 2. SEM images of longitudinal view of PLGA fibres with HAp and TCP after 20 weeks in PBS.

TABLE 1. Fibres tenacity, intrinsic viscosity and mass change after 20 weeks of degradation in PBS.

Polymer	Type of nano-additives	Tenacity decrease [%]	Intrinsic viscosity decrease [%]	Mass increase [%]
PLDLA	-	57%	18%	14%
	HAp	60%	19%	15%
	TCP	70%	23%	11%
PLGA	-	75%	52%	8%
	HAp	91%	61%	13%
	TCP	61%	51%	11%

Conclusions

Fibres properties as well as progress and intensity of degradation depend mainly on the presence of HAp or TCP into a fibrous matrix. Their presence effect into a tenacity decrease and acceleration of the degradation.

Acknowledgments

The paper presents results of the studies conducted in the frame of PhD Thesis of Paulina Król [7], which were carried out in Department of Material and Commodity Sciences and Textile Metrology and Department of Man-Made Fibres of Lodz University of Technology.
The research was financed by National Science Centre project no. 6258/B/T02/2011/40. The fibres were obtained in Department of Material and Commodity Sciences and Textile Metrology and Department of Man-Made Fibres of Lodz University of Technology.
Authors would like to thank to dr hab. inż. Teresa Mikołajczyk, prof. PŁ for her contribution, substantive support and cooperation.

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SILOXANE LAYERS MODIFIED WITH CARBON NANOFORMS FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 148 (2018) 114]

Introduction

One of the key problems of the biomaterial engineering is research in the field of surface modification of implantable materials. Layers deposited on metallic surfaces preserves from diffusion of corrosion products to the biological environment. Moreover, they provide required cell and tissue response on the implant inserted into the organism of the patient, appropriate to the particular application. Implants in the bone surgery, besides no corrosivity, should be characterised by bioactivity stimulating the osteosynthesis process. Whereas, implants for cardiac surgery should have non-thrombogenic properties of the surface to prevent pathological mineralization.

Therefore, functional biomaterials responding to the medicine demand might be obtained by the modification of the surface of the implantable material. It might be accomplished by deposition of the specified top layer. It is well known, a formation of materials with the use of methods and tools of nanotechnology might take place on two levels, namely by the modification of the nanoparticle itself or by the application of nanoparticle as the modifier of the particular material.

This second approach in mostly eliminates any danger resulting from nanoparticle toxicity and nowadays is considered as the effective way of fabrication of new materials with properties desired from the medical point of view[1,2].

Carbon nanoforms, such as multiwalled carbon nanotube (MWCNT) and graphene oxide (GO), ale materials of great efficiency in properties modification of biomedical material. The aim of the study was preparation and examination of nanocomposite layers based siloxanes modified with the use of carbon nanoforms. Interactions between carbon nanoforms and polymeric matrix modify the structure of the layer on the molecular level. Moreover, they give new unique properties, impossible to obtain in other ways. Furthermore, implementation of carbon nanoforms into the polymer gives the coating unique nanotopography – element of the structure essential in the contact with cells and biological environment. By appropriate selection of the type of functionalization of nanoparticles and by control of polymer structure (carbon/silicon atoms ratio) it is possible to obtain materials of differential properties: non-thrombogenic, germicidal as well as osteogenic.

Materials and Methods

Nanocomposite layers were obtained on the titanium surface with the use of electrophoretic co-deposition. The stable suspension of polysiloxane and functionalized MWCNTs in ethanol alcohol was prepared and applied in the deposition process.

The study included structural analyses using infrared and Raman spectroscopy as well as material testing in terms of corrosion resistibility and adhesion to the substrate. Biological tests referred to the *in vitro* bioactivity of the chosen materials determined in the so-called Kokubo test and cell examination in terms of proliferation and differentiation of NHOst cell cultures.

Results and Discussion

The produced layers are characterized by good adhesion to the substrate, they have isotropic fibrous topographies. SEM images (FIG. 1) depicted that siloxane uniformly coated MWCNTs and biomimetic morphology was obtained.

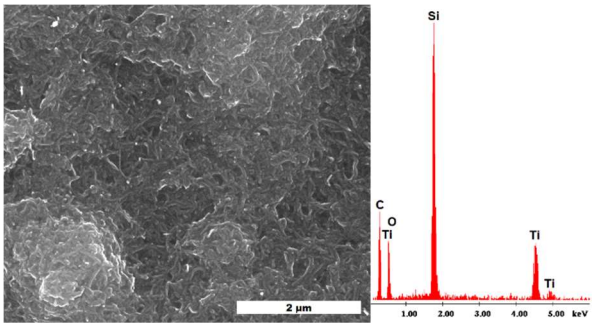

FIG. 1. SEM image and EDS analysis of the siloxane/MWCNTs nanocomposite layer

Spectroscopic examination confirmed desired phase and chemical composition. In *in vitro* tests, both bioactivity and biocompatibility were proven. After so-called Kokubo test hydroxyapatite was discovered on the sample surface. The cell activity was on the satisfactory level

Conclusions

Our research shows that that the modification of polymeric materials (siloxanes) with the use of carbon nanoforms is the effective method leading the formation of multifunctional materials characterized by elevated anticorrosive parameters such as good adhesion to the substrate. By suitable control of the layer components in terms of its chemical composition (functional groups on the carbon nanoparticle surface, the structure of polymeric matrix – C/Si ratio) it is possible to obtain coatings of high biocompatibility and bioactivity.

Acknowledgments

This work has been supported by the National Science Centre project No. 2017/25/B/ST8/02602.

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CURRENT CONCEPTS OF ARTICULAR CARTILAGE REPAIR

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[ENGINEERING OF BIOMATERIALS 148 (2018) 115]

Introduction
Osteoarthritis (OA) is the most common form of joint disease, affecting mainly the load bearing joints. It can lead to severe disability and loss of quality of life. A huge number of surgical strategies aimed at cartilage repair is currently available including micro-fracture, osteochondral autograft transplantation, mosaicoplasty and osteochondral allografts, autologous chondrocyte implantation (ACI). Good clinical outcomes of such procedures are strongly influenced by the size of the chondral lesion, proper mechanical loading and stability of the affected joint and the presence of coexisting joint pathologies. Because of that the choice of optimal surgical treatment of chondral defects remains highly controversial and has yet to be determined. We present a systemic approach of managing OA based on our own experiences and the review of current literature.

Acknowledgments
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COMPOSITE COATINGS OF POLYLACTIDE WITH GRAPHENE OXIDE AND HYDROXYAPATITE ON TITANIUM

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[ENGINEERING OF BIOMATERIALS 148 (2018) 116]

Introduction

The surface is the most important part of an implant, due to the fact that it is the one that will always be in contact with the living tissues. Almost all of the interactions between cells and tissues with a material at the tissue implant interface refer to surface phenomena, thus a surface plays an important role in the integration of the implant with surrounding tissues. In order to improve the biological properties of titanium, multifunctional composite coatings are used to modify surface properties [1-3].

Materials and Methods

In this work, coatings were composed of polylactide (PLA, PL38 Purasorb, Purac), graphene oxide (GO, ITME) and hydroxyapatite (HAP, Chema Elektromet). Four compositions were prepared: PLA, PLA with 3wt% of GO, PLA with 1wt% of HAP and PLA with 3wt%of GO and 1wt% of HAP. Thin films were deposited on pre-etched [3] commercially available pure titanium Grade 2 plates (10x10mm) (Torresin Titanio SRL, Italy). Pure PLA (solution: 1g PLA / 10ml DCM) and PLA with additives (homogenized with sonicator, Vibra-Cell Sonics) were deposited by dip coating method with a speed of 50mm/min. The surface roughness (R_a, R_t, R_z) was measured using profilometer (Hommelwerke T 4000). For evaluating the quality of the coatings, scratch tests (NST 50-146 CSM Instruments) and microscopic observations (digital microscope VHX 5000, Keyence and scanning electron microscope FEI Nova NanoSEM 200) were carried out. Scratch tests were used for analysing the adhesion of the coatings to the titanium. Adhesion force was determined by generating a controlled scratch with a diamond tip on the sample under a progressive load perpendicular to the surface. The maximum load on the indenter was 100 mN.

Results and Discussion

As it is shown in FIG 1, the surface roughness was higher for composite coatings than for pure polymer layer. HAP powder presence had higher impact than GO. The highest R_a, R_t and R_z parameters values were for PLA-GO/HAP. Microscopic observations (FIG. 1b) did not show detachment of the composite coatings in a result of scratch test. The polymer modified with graphene and/or hydroxyapatite exhibited plastic deformation, it was pressed in and pulled with a movement of a scratch tester tip. It is clearly visible on the SEM images, eg. for a PLA-GO coating (FIG. 2). Similar behaviour was observed for other composite coatings. In the case of pure polymer coating, even with lower magnifications (200x), localized changes after scratch were visible. In FIG. 1a, arrows point to spots were film is no longer transparent and starts loosing contact with a titanium surface. It was confirmed with SEM observations that showed discontinuity of the polymer layer, its detachment and folding (FIG. 2a).

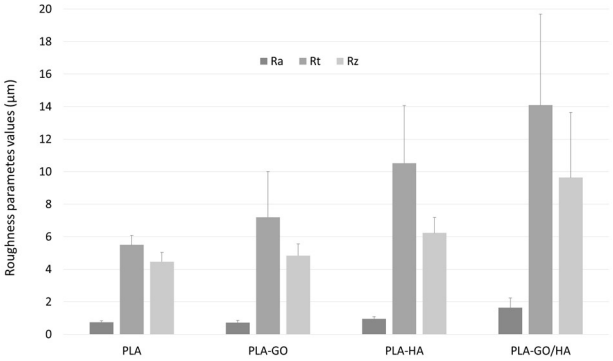

FIG. 1. The surface roughness of the titanium with coatings.

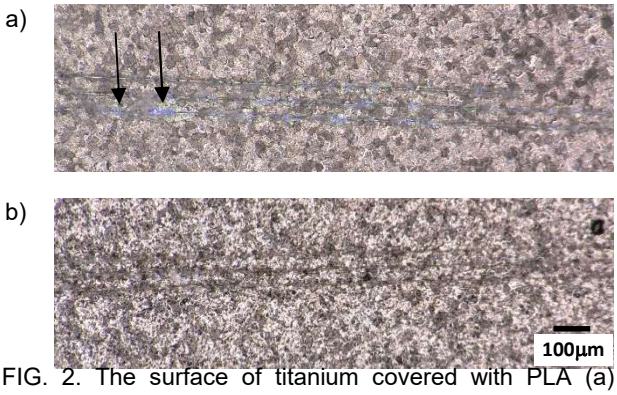

FIG. 2. The surface of titanium covered with PLA (a) and PLA-GO (b) after the scratch test (optical microscope).

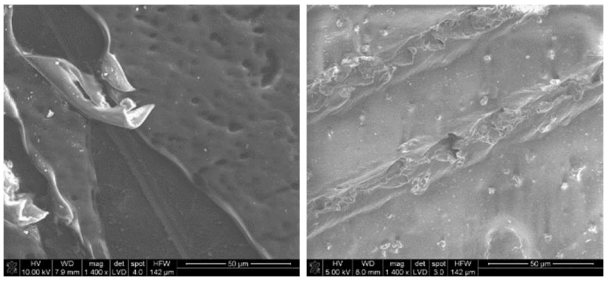

FIG. 3. SEM image of titanium covered with PLA (a) and PLA-GO (b) after the scratch test.

Conclusions

Dip coating method allowed obtaining continuous and good quality PLA and PLA with GO and/or HAP composite coatings. The adhesion of the composite films to titanium was better than that of pure polymer, what may be related to nanocomposites interactions with titanium surface or polymer plastification by additives [4].

Acknowledgments

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IMPROVED ADHESION AND GROWTH OF VASCULAR SMOOTH MUSCLE CELLS ON POLYCAPROLACTONE NANOFIBROUS MEMBRANES MODIFIED BY AMINE-RICH PLASMA

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[ENGINEERING OF BIOMATERIALS 148 (2018) 117]

Introduction
Plasma polymerization of amine rich coatings is a promising technique for preparing cell carriers for tissue engineering. This technique can be used for modifying a wide range of biomaterials, such as synthetic polymers, ceramics and metals [1, 2], and has been shown to improve the adhesion and growth of various cell types, e.g. osteoblasts [1] and vascular endothelial cells [2]. In this study, we have explored plasma polymerization of cyclopropylamine (CPA), i.e. a promising isomer of allylamine, on electrospun polycaprolactone (PCL) nanofibrous membranes. The modified membranes were then tested with vascular smooth muscle cells (VSMC), i.e. an important cell type used in advanced vascular tissue engineering aiming at the reconstruction of the *tunica media* in vascular replacements.

Materials and Methods
Plasma polymers were deposited from CPA in low pressure radio frequency (RF) discharges, as described earlier [3]. The average power ranged from 9.9 W to 150 W. The freshly modified nanofibrous membranes were cut into square samples 1x1 cm in size, fixed in CellCrown inserts (Scaffdex), and inserted into polystyrene 24-well cell culture plates (TPP). The membranes were then seeded with VSMCs (isolated by explantation from the thoracic aorta of young male Wistar rats) in a density of 17,000 cells/cm², and in 1.5 ml of DMEM medium with 10% foetal bovine serum (Gibco). The cells were cultured for 1, 3 or 7 days, and were counted on microphotographs. For each experimental group and time interval, three samples were used.

Results and Discussion
The numbers of initially adhered VSMCs on day 1 after seeding ranges from 1550 ± 150 to 6200 ± 650 cells/cm², and were higher on all modified membranes than on pure PCL membranes. This trend remained the same for the rest of the experiment. The cells on the modified PCL membranes were similar in shape to the cells on the control polystyrene wells, i.e. they were spindle-shaped and polygonal, while the cells on the pure PCL membrane were mostly rounded (FIG. 1). The number of VSMCs was rising continuously with the time of the experiment. We also observed a positive correlation of the cell number with the average power. On day 7, the lowest cell number (3350 ± 460 cells/cm^2), which was only slightly higher than that on unmodified PCL (2930 ± 200 cells/cm^2) was obtained on the sample modified at the average power of 9.9 W, while the highest cell number (7200 ± 860 cells/cm^2) was achieved on the sample modified at the average power of 150 W. This result was rather surprising, because the content of amine groups, which are known to improve cell adhesion, was inversely correlated with the average power. However, the samples modified at the highest average power are more crosslinked, i.e. more stable and less soluble in the water environment [3]. The amine groups improve the cell adhesion mainly by their positive charge, which promote the adsorption of cell adhesion-mediating molecules, e.g. fibronectin and vitronectin, in appropriate geometrical conformations, which increases the accessibility of specific amino acid sequences in these molecules for adhesion receptors on cells [4]. Similar results were obtained after grafting silicon substrates with CPA using ultraviolet light, which improved the adhesion of epithelial cells of three lines [5].

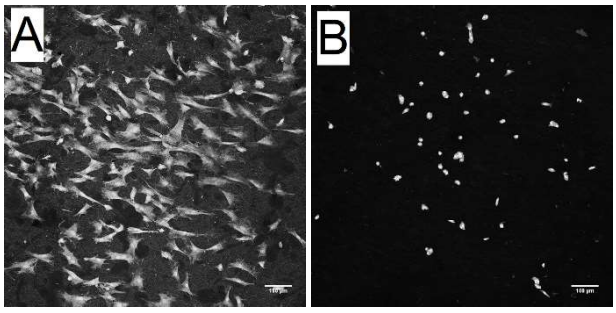

FIG. 1. Morphology of vascular smooth cells on day 7 after seeding on sample modified at average power of 33 W (A) or pure PCL membrane- (B). The cells were stained with Texas Red C2-maleimide and Hoechst #33342. Leica TCS SPE DH 2500 confocal microscope, obj. 10.0x0.30, bar = 100 µm.

Conclusions
The modification of PCL nanofibrous membranes by plasma polymerization of CPA had positive effect on the adhesion and growth of VSMCs. This result indicates that this technique is perspective for modification of the materials used for construction of vascular replacements.

Acknowledgments
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MODYFICATION OF ELECTRIC PROPERTIES OF HYDROXY-APATITE WITH MAGNETITE - PRELIMINARY RESEARCH

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[ENGINEERING OF BIOMATERIALS 148 (2018) 118]

Introduction

Hydroxyapatite has been widely used clinically for bone defects treatment. Its chemical composition and mechanical properties are similar to a natural bone tissue. Besides surface and structural modifications improving healing processes, characteristics of HAp can be tailored by a composite preparation. Depending on needs, mechanical, chemical, biological or electric properties can be altered with different additives. It is well known fact that a bone for growing is stimulated by external forces. Moreover, it has the ability of generating electrical charge when stressed. It is said that these two factors are the most important for controlling the configuration of a bone structure in accordance with the Wolff's law.

The aim of this study was to investigate the influence of magnetite nanoparticles (MGT) addition on hydroxyapatite electrical properties under compression.

Materials and Methods

For raw material composite HAp nanopowder (Chema Elektromet) with magnetite nanopowder (Sigma Aldrich) was combined and homogenized by shaking. Three compositions were prepared: A) HAp + 0,5 vol% MGT; B) HAp + 1,0 vol% MGT e; C) HAp + 1,5 vol% MGT. Cylindrical-shape samples (d = 12mm, h = 16,5mm) were made by isostatic pressing (5 MPa) in room temperature. Raw samples were sintered in 1000°C. Microscopic observations were made (optical x20 – x100 KEYENCE VHX5000 and SEM x70 – x500 000 FEI NOVA NANO SEM 200). Mechanical properties were described in uniaxial compression test Zwick-1435, V = 0,2 mm/min. For electrical changes specially designed and fabricated circuit consisted of two copper plates wired with 9V DC battery and digital multimeter (UNI-T UT33C) was fabricated. Copper plates were attached to the top and bottom surface of sample, then placed between pressure pads of testing machine and compressed. The voltage was measured and recorded.

Results and Discussion

Microstructure analyzes show that the grade of homogeneity is not satisfying. There are aggregates of bulk HAp surrounded probably with HAp-MGT composite – pinkish in colour (FIG. 1a), however in SEM pictures magnetite nanoparticles were barely visible (Fig.1b). For all types of samples – different vol% of MGT (A, B and C) similar effect was observed. Mechanical testing revealed that addition of 1,0 vol% of MGT significantly improved compressive strength (FIG. 2) and electrical conductivity (pure HAp: R_c = 18 MPa, V = 4,5-5V; HAp + 1,0 vol% MGT: R_c = 28 MPa, V = 8-9V). For all types of composites no piezoelectric effect was detected.

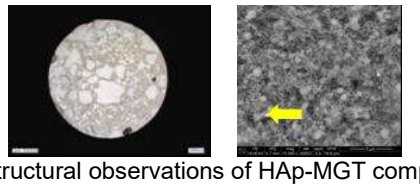

FIG. 1. Structural observations of HAp-MGT composite. a) x20; b) x10 000 (arrow – magnetite).

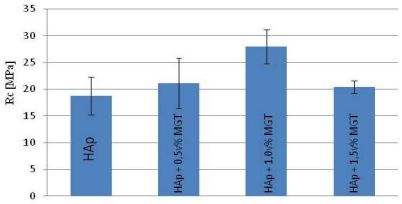

FIG. 2. Compressive strength of tested materials.

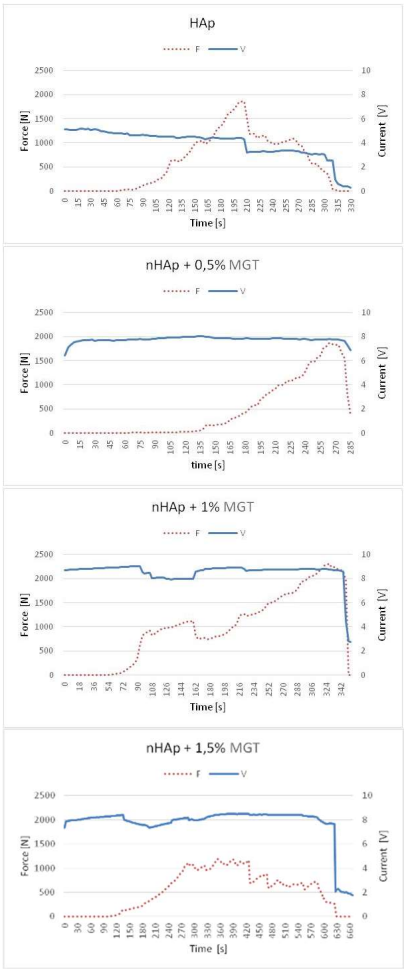

FIG. 3. Changes in electrical conductivity of different composites under compression.

Conclusions

Magnetite nanopowder can be used as a HAp properties modifier. Depending on the quantity both mechanical and electrical characteristics can be tailored in wide range. Surprisingly, the presence of HAp agglomerates didn't decrease compression strength.

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POTENTIAL OF ELECTRO-SPINNING TECHNIQUE TO DRUG DELIVERY SYSTEM

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[ENGINEERING OF BIOMATERIALS 148 (2018) 119]

Introduction

Electrospinning is technique used to manufacturing nano- and submicrofibers base on synthetic or natural polymers. The size and microstructure of fibrous scaffold are suitable to cells adhesion and proliferation. Microstructure of fibrous scaffold mimicking natural extracellular matrix (ECM) [1]. Additionally, biomaterials used to electrospinning procedure can be modified by bioactive compound, peptides or growth factors (FIG. 1.). The non-woven materials characterized by high porosity and high surface area to volume, which allow for efficient loading of the material with drug. It is also a possibility to obtain a porosity of the single fiber. That can further enhance the surface area to volume ratio and increase the functionality of the fibres [2].

This work was based on achievement a porous fibrous membrane with antibacterial properties. For this purpose we obtained three different fibrous membranes by electrospinning using polylactide (PLA) and three sets of solvents. The main goal was to achieve porosity of single fiber to get better antibacterial properties in the next step. We solved this issue using high humidity during electrospinning process and introducing gentamicin into the spinning solution. The obtained porosity can be studied using temporometry based on changes in heat of fusion.

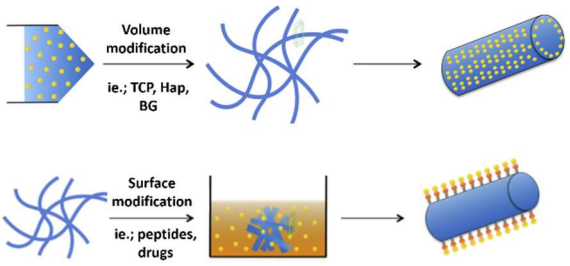

FIG. 1. Electrospinning fibers modification: on the surface and in the bulk.

Materials and Methods

The PLA 3251D polylactide from Nature Works was used in the research as base material for electrospinning. Analytically pure reagents provided by Avantor SA: dichloromethane (DCM), dimethylformamide (DMF), chloroform (CHL) and dimethyl sulfoxide (DMSO) were used as solvents for the preparation of spinning solutions. Porous polymer fibers were modified with 5% w/v of gentamicin (Polfa SA), in the form of gentamicin sulfate. The electrospinning process was carried out under experimentally determined conditions, namely: humidity (30-70%), temperature (25°C) and voltage (12-15 kV). Both the measurement of diameters and the microtexture observations of PLA submicrofibers were made using the scanning electron microscope Merlin Gemini II (Zeiss). Surface wettability of the tested materials was determined by means of direct measurements (DSA 10, Kruss) at room temperature using high purity water (UHQ, PURE Lab, Vivendi water) as a measuring liquid. The free surface energy was determined by the Owens-Wendt method using diiodomethane as a non-polar liquid.

Experiments on controlled drug release were performed using small pieces of electrospun membrane (10 mg) that were incubated in 50 ml of phosphate buffer saline (PBS) medium into polypropylene tubes at 37°C for 7 days. The concentration of drug in the immersion medium was measured by plasma inducted spectroscopy (ICP-ASA, Hewlett-Packard ICP 4500 spectrometer).

Results and Discussion

The results of the conducted research indicate that porous and non-porous PLA fibers can be successfully modified with gentamicin. The different size (diameter) of the fibers influenced the pore size (FIG. 2). Indirect proof for the presence of gentamicin are results of wettability tests (CA). Non-woven materials with gentamicin characterized low wettability in comparison to porous fibers but without antibiotic. The homogeneous pore size distribution in membrane with porous fibers well corresponds to the shape of the drug release profile. The release profile has a much milder course, when the fibers size and the pores diameter were larger (with larger surface pores). Slower kinetic of drug release was observed when the diameter of fibers and pores had nanometric diameter.

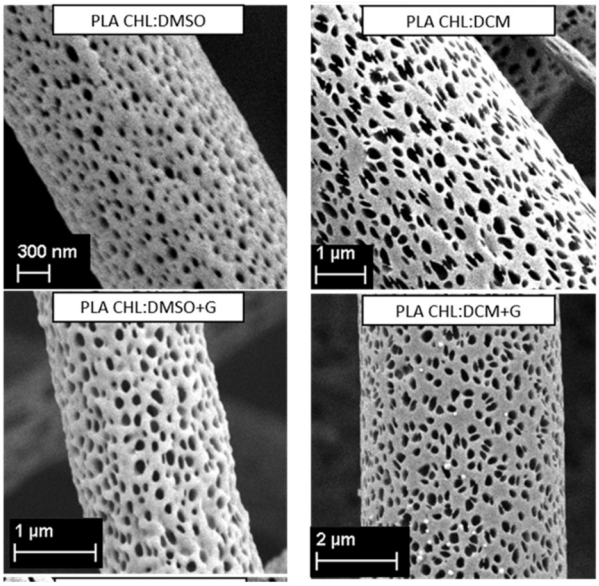

FIG. 2. Morphology of porous fibers with and without gentamicin.

Conclusions

The preliminary results show, that it is possible to propose several solutions of materials with optimal properties, designed on the basis of a correct combination of porous and solid fibers, which will ensure an prolonged release time of the drug.

Acknowledgments

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THE EFFECT OF FUNCTIONALIZED HAp ADDITION ON THE SELECTED PROPERTIES OF POLYACETAL-BASED COMPOSITES

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[ENGINEERING OF BIOMATERIALS 148 (2018) 120]

Introduction

Polyacetal (POM, $-(CH_2-O)_n-$) is a linear, thermoplastic polymer that exhibits a high crystallinity (45-85%). Consequently, POM has high tensile strength, shear strength, stiffness, and toughness, good dimensional stability, chemical and abrasion resistance as well (1). Hydroxyapatite (HAp), as a bioactive ceramic, has the ability to form chemical bonds with living bone tissue. HAp can be easily incorporated into POM matrix in order to obtain the bioactive, durable composite materials. However, pure HAp induces the thermal degradation of POM matrix during the processing of composites (2). Using an organically functionalized HAp instead of pure one is an effective way to prevent this undesired behavior of POM (3). Moreover, the presence of the organic part on the nanoparticles surfaces increases the compatibility of the inorganic particles with the polymeric matrix (4).

Materials and Methods

The objective of this work was to fabricate the polyacetal-based composites containing organically functionalized hydroxyapatite (OF-HAp) and to characterize their selected properties.
Polyacetal copolymer (Ultraform®, BASF) was used as a composites matrix. Hydroxyapatite nanoparticles (HAp, nGimat Co) were organically functionalized with poly(ethylene glycol) (PEG 2000, Sigma Aldrich) and 1,6-hexamethylene diisocyanate (HDI, Sigma Aldrich) as a coupling agent. Dibutyltin dilaurate (DBTDL, Sigma Aldrich) as the catalyst and anhydrous n,n-dimethylformamide (DMF, Avantor) as the solvent were used in the synthesis.

Processing of POM/OF-HAp composites

In the first step, POM and OF-HAp powders were dried for 2 h in 80°C. Then, POM and OF-HAp were mechanically mixed (0, 1.0, 5.0, and 10.0%, calculated in relation to pure HAp) and extruded in a twin-screw extruder (50 rpm, 210°C). Finally, the composites were shaped by injection moulding method.
Next, the composites were investigated using TG and DSC techniques. The AFM microscopy and the Brinell hardness test were also applied.

Results and Discussion

Thermogravimetric (TG) analysis confirmed that OF-HAp nanoparticles contain about 25% of organic part. There was no decrease in the thermal stability of POM/OF-HAp composites. Furthermore, the composites containing OF-HAp exhibit higher decomposition temperature in comparison to pure POM that is a very desirable effect.

DSC results showed small increase in the crystallinity of POM in POM/OF-HAp composites. The biggest increase of 5% was observed for samples containing 5.0% of OF-HAp. It can suggest that OF-HAp acts as a nucleating agent for POM. This observation was also confirmed by AFM microscopy. As can be seen from FIG. 1, the incorporation of OF-HAp affects the size of spherulites in POM/OF-HAp nanocomposite and higher number of spherulites are formed as compared to pure POM.

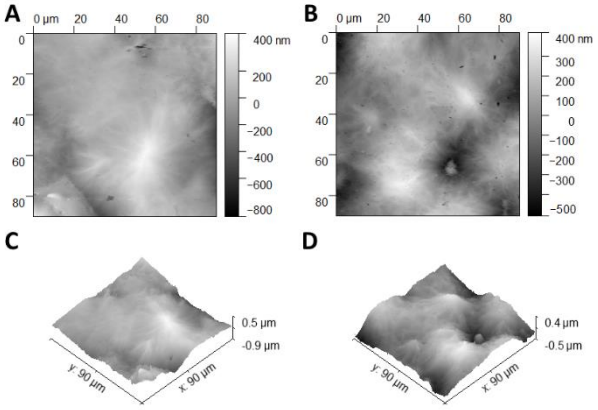

FIG. 1 AFM images of POM (A,C) and POM/1.0% OF-HAp composite (B,D).

Hardness test confirmed that all composites displayed higher hardness than pure POM, but the biggest increase was observed for POM/5.0% OF-HAp. This phenomenon was most probably the effect of increase in the crystallinity of POM.

Conclusions

In this work high strength polyacetal-based materials modified with organically functionalized, bioactive bioceramics were obtained. From the foregoing results it can be seen that the presented materials have great potential as non-resorbable biomaterials intended to the regeneration of a large bone defects.

Acknowledgments

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