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THE PROMISE OF COMPOSITE POLYMERS FOR BONE TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 7]

Abstract
The repair of bone defects is of particular interest for orthopaedic, oral, maxillofacial, and dental surgery. Bone loss is conventionally reconstructed by bone grafting. Depending on size and location of the defect, this method has limits and risks. In addition, in the context of reconstruction of the craniofacial skeleton after radiation therapy, we need to improve therapeutic options for patients suffering from such disastrous sequelae of radiation therapy.
While the use of BMPs has been approved for bone regeneration applications, their use is contraindicated in a carcinological context, due to concerns that these anabolic growth factors may contribute to tumor cell proliferation.
Moreover, the main limitations are to regenerate a functional vasculature [1] and to restore bone innervation that also played a major role for bone tissue regeneration [2,3].
In such context, biomaterials such as calcium phosphate matrices, free of reparative cells, cannot offer sufficient potential for supporting especially vascularization of newly formed bone. Polymers and mainly composite based-polysaccharides, because of their versatility, their possible supplementation with a mineral phase (i.e hydroxyapatite particles), have immense potential for mimicking bone tissue, by trapping osteogenic and angiogenic factors and then promoting both osteogenesis and angiogenesis [4,5].
The other challenge in the field of bone tissue engineering is to favour anchorage of sensory neurons within 3D matrices that could produce neurotrophic factors [6], activate the coupling of osteogenesis and angiogenesis.
Here, we will describe a cell-free approach for bone tissue engineering [7] using injectable composite polymers, their *in vitro* and *in vivo* validation in preclinical models from small to large animals. We will also show how composite polymer chemistry can also favour cell interactions between mesenchymal stem cells, endothelial cells and stimulate bone tissue regeneration.

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MINERALIZED HYDROGELS FOR BONE REGENERATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 8]

Abstract

Biomaterials for bone regeneration have predominantly been fabricated from inorganic substances such as various forms of calcium phosphate (CaP), e.g. hydroxyapatite, tricalcium phosphate and brushite. CaP materials are mechanical stable and bioactive, i.e. they form a direct bone with surrounding bone tissue. However, such pure CaP materials have certain drawbacks. They are brittle, difficult to handle in granulate form and difficult to shape in block form. Furthermore, the incorporation of biologically active substances is not easy.

Hydrogels are highly hydrated three-dimensional polymer networks that are formed by crosslinking of polymer chains in solution. Hydrogels have been widely used as vehicles for drug delivery and are being used increasingly as biomaterials for tissue regeneration. As their main component is water, they have many advantages over pure inorganic materials. Firstly, the incorporation of water-soluble biologically active substances to promote tissue growth (e.g. growth factors) or to combat infection (e.g. antibiotics) is straightforward. Secondly, they are much less brittle. Thirdly, they can be implanted in a minimally invasive manner by injection, as they can undergo gelation, i.e. the transition from liquid to solid, after injection. However, their main disadvantage also stems from the fact that the mail component is water: hydrogels are mechanically weak.

In order to combine the advantages of inorganic and hydrogel biomaterials, attention has recently been focused on the development of composites on the basis of mineralized hydrogels. Several strategies have been tried [1].

The most common strategy is the addition of preformed inorganic particles to the polymer solution before gelation, after which the particles remain entrapped in the crosslinked polymer network. Ideally, the particles can be distributed homogeneously in the hydrogel. The gelation process can be induced by addition of inorganic particles. For example, the addition of bioactive glass particles to a solution of the anionic polysaccharide gellan gum results in hydrogel formation due to release of ions from the particles [2]. In other words, the particles serve as an "ion-delivery system" to provide homogeneous gelation.

Another strategy is to promote precipitation of the inorganic phase in the hydrogel by increasing the concentration of ions. This can be achieved biomimetically using the enzyme alkaline phosphatase (ALP) which is responsible for the mineralization of bone tissue *in vivo* by cleaving phosphate ions from organophosphate and thus increasing the local phosphate concentration, which in turn promotes CaP precipitation [3].

Yet another strategy is the incorporation of calcium- or phosphate-binding molecules in the hydrogel, in order to increase local ion concentrations and promote CaP precipitation. Once such biomolecule is polydopamine, which binds calcium ions [4].

An added flexibility of mineralized hydrogels is the possibility of manipulation of either the hydrogel phase, or the inorganic phase, or both. For example, in the case of a hydrogel mineralized with CaP, the inorganic phase may be modified by incorporation of magnesium in order to promote adhesion and proliferation of bone-forming cells [5], or by incorporation of zinc in order to endow antibacterial activity [6]. Alternatively, the hydrogel phase may be modified by incorporation of biologically active molecules such as polyphenols, which both bind calcium ions and exhibit antibacterial activity [7].

Mineralization strategies will be illustrated on the basis of previous work [1-7].

Acknowledgments

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DESIGN AND PRECLINICAL VALIDATIONS OF POLYSACCHARIDE-BASED NANO/MICRO/MACROSYSTEMS FOR TISSUE ENGINEERING AND MOLECULAR IMAGING

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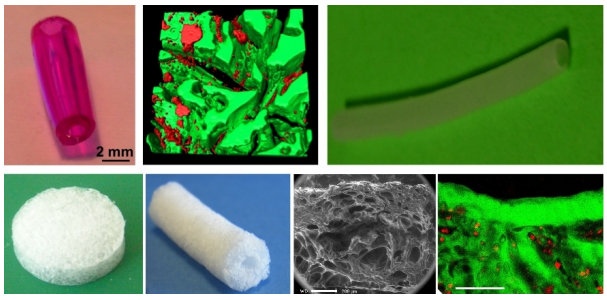
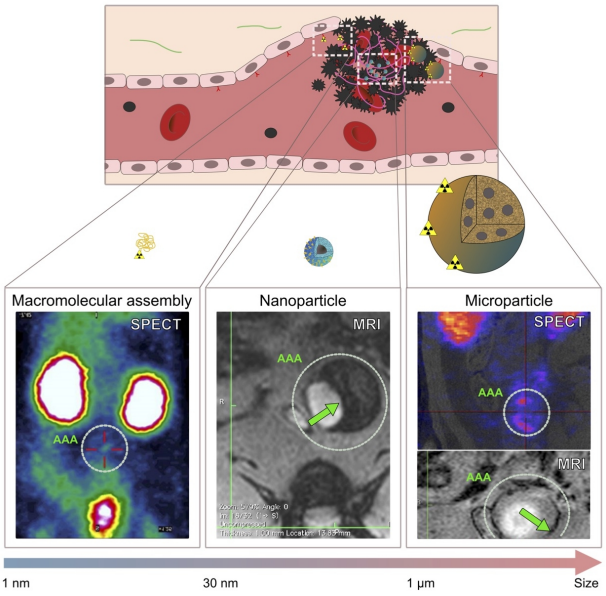
[ENGINEERING OF BIOMATERIALS 138 (2016) 9]

Abstract
This presentation intends to present polysaccharide-based matrices for regenerative medicine and as drug delivery systems and targeted contrast agents for molecular imaging.
One main challenge of tissue engineering is to create an optimal environment for growing therapeutic cells to regenerate damaged tissues. This environment can be reconstituted by using 3D matrices, in which cells can be organized into a tissue-like structure. We have prepared polysaccharide-based porous matrices having controlled pores and porosity for several cell types. These porous hydrogels made of natural biodegradable and biocompatible polysaccharides have architectural characteristics adapted to the cell culture in 3D.
We have developed them to different shapes and sizes. Further studies have demonstrated the performance of these matrices for tissue repair in vitro as well as in small and large animals. Examples for heart, vessel, and bone will be presented.



Moreover, polysaccharide-based nano and microsystems were also designed and used for the imaging of cardiovascular pathologies as targeted contrast agents for molecular imaging. Examples will be provided using several types of imaging modalities for thrombus detection.



We will also present how to use nanomaterials for regenerative medicine. Indeed, adhesion by aqueous nanoparticle solutions can be used in vivo to achieve rapid and strong closure and healing of deep wounds in rat skin and liver. Nanoparticles can also be used to fix polymer membranes to tissues even in the presence of blood flow, such as occurring after liver resection, yielding permanent hemostasis within a minute. Furthermore, medical devices and tissue engineering constructs could be fixed to organs such as a beating heart.

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TISSUE ENGINEERING IN RECONSTRUCTIVE UROLOGY, DREAMS AND REALITY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 10]

Introduction
Tissue engineering and biomaterials science currently offer the technology needed to replace the urinary tract wall and kidneys. This review addresses current achievements and barriers for the regeneration of the urinary tract and kidney.

Materials and Methods
Medline was search for urinary tract tissue engineering, regenerative medicine and stem cells. In this review we analyzed history of urinary tract tissue engineering together with current attempts in urinary tract elements construction using tissue engineering methods. Based on literature and our own experience we presented problems and future perspectives related to the artificial urinary tract elements.

Results and Discussion
The availability of kidney and other organs from matching donors is not enough for many patients on demand for organ transplant. The most important achievements in the field of regenerative medicine of kidney, which were mentioned and described here, are currently cumulated in 4 areas of interest: stem cell-based therapies, neo-kidneys with specially designed scaffolds or cell-seeded matrices, bioartificial kidneys and innovative nanotechnologically bioengineered solutions. Regenerative medicine is still insufficient to completely replace current therapy methods used in patients with chronic kidney disease [1]. Large ureter damages are difficult to reconstruct. Current techniques are complicated, difficult to perform, and often associated with failures. The ureter has never been regenerated thus far. Therefore the use of tissue engineering techniques for ureter reconstruction and regeneration seems to be a promising way to resolve these problems. For proper ureter regeneration the following problems must be considered: the physiological aspects of the tissue, the type and shape of the scaffold, the type of cells, and the specific environment [2,3]. Numerous studies to develop a substitute for the native urinary bladder wall using the tissue engineering approach are ongoing. The idea of urinary bladder regeneration through tissue engineering is an old one. Many natural and synthetic biomaterials were used for urinary bladder regeneration with a wide range of outcomes. Stem cells combined with biomaterials open new treatment methods, including even de novo urinary bladder construction. Recent progress in the tissue engineering field suggest that in vitro engineered bladder wall substitutes may have expanded clinical applicability in near future but preclinical investigations on large animal models with defective bladders are necessary to optimize the methods of bladder reconstruction by tissue engineering in humans [4,5]. There are still many issues before advances in tissue engineering should be introduced before clinical application [6]. One of the most important is stem cells aging and their application for urinay bladder reconstruction [7]. Expression of cytokines and MMPs during bladder regeneration can influence the final result [8]. The histological presence of a regenerated all layers of the urinary bladder do not guarantee proper urinary bladder function [9]. Urine is a highly cytotoxic agent, which influences stem cell therapies in urology [10]. Finally, stem cells harvest from oncological patients carry potential risk cancer development after regenerative therapy [11]. Artificial urinary conduit has a great chance to become the first commercially available product in urology constructed by regenerative medicine methods [12].

Conclusions
Numerous studies to develop a substitute for the urinry tract elements using the tissue engineering approach are ongoing. Stem cells combined with biomaterials open new treatment methods. Before tissue engineering techniques could be recognize as effective and safe for patients, more research studies performed on large animal models and with long follow-up are needed to carry on in the future.

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FE ANALYSIS OF THE SHOULDER CERAMIC PROSTHESIS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 11]

Introduction

Damages of the shoulder joint lead to painful and restricted movement. Efficacy in relieving pain and restoring functions of the joint lead to high popularity of the prosthesis. This work deals with a static FE analysis of the influence of a load in a human shoulder on a ceramic humeral head with and without ribs. In order to use a finite element analysis as a successful tool in a shoulder prosthesis development, the validated input forces are important.

The most cited source of data on a load of a shoulder joint was for many years a study of Poppen and Walker [1]. Based on 2D model they have determinated a force of 90% BW in scapula plane for normal abduction. To a certain point a breaking study was in-vivo telemetric measurement in shoulder endoprosthesis published by Bergmann [2], later by Westerhoff [3].

Materials and Methods

These are the results of Bergmann [2] which were used in this finite element analysis of the shoulder endoprosthesis. The Bergman's results are accessible online on Orthoload [4].

As the movement, the abduction test in standing position has been chosen. A 72 years old man of 83 kg elevated a 2 kg load up to 120 deg. The experimental results of Bergman are illustrated on FIG. 1. The resulting force of 1760 N was applied to the FE model in reference point coupled to outside glenoid surface.

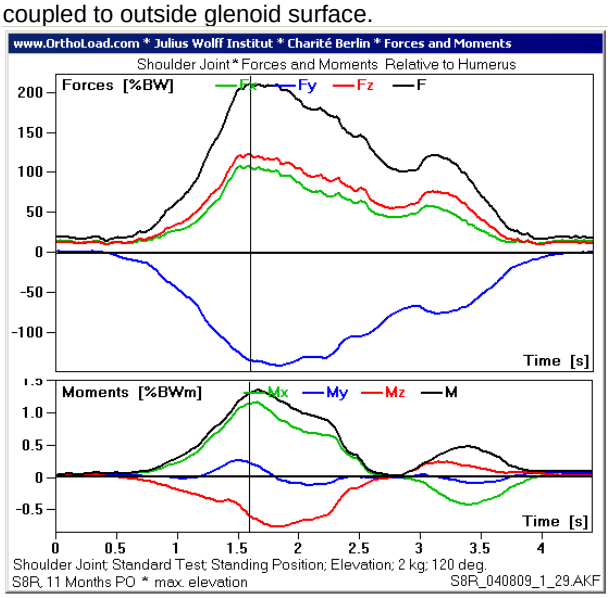

FIG. 1. Load of shoulder [4].

Geometric and finite element model (C3D10M elements) of upper limb with the implanted shoulder endoprosthesis are illustrated in the following FIG. 2 as well as the detail of two ceramic humeral heads (made of ZrO_2, with and without ribs) which were analysed in this work.

The following parts of the endoprosthesis were calculated in the analysis: ceramic head with/without ribs, neck, neck insert, humeral shaft, humerus and simplified glenoid.

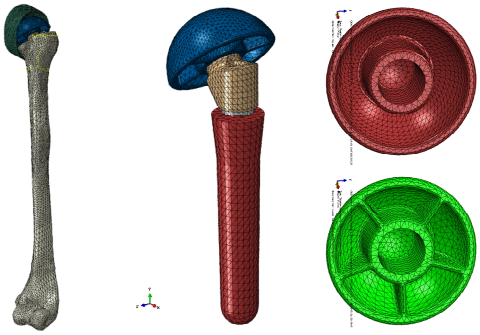

FIG. 2. Geometry model, details of humeral heads.

Results and Discussion

The load applied into the model was adopted from the experimental study presented in OrthoLoad [30] database. The static force of 1760 N has been chosen to prove the appropriate geometry of the humeral head in both constructions. See the FIG. 3 for the stress distribution (according to HMH theory) on the surface of both ceramic humeral head. The maximal stresses of 58 MPa resp. 33 MPa demonstrates the right construction solution of the head with resp. without ribs at least in the abduction test used in this study.

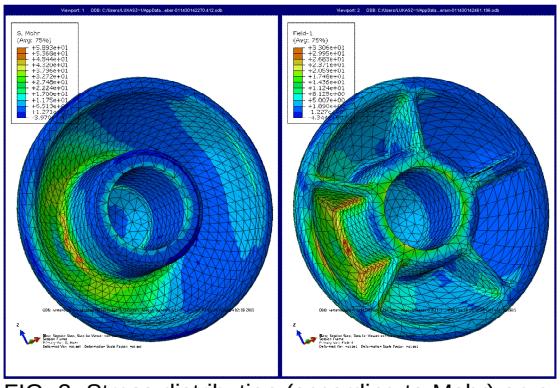

FIG. 3. Stress distribution (according to Mohr) on surface of ceramic humeral head [MPa].

Conclusions

The aim of this work was to validate the construction of ceramic humeral head with and without ribs. The results proved the right construction of both zirconia heads. Since the maxima of stresses according to Mohr are deeply below the strength of the material, the challenging problem is the manufacturing of the endoprosthesis.

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COLLAGEN MATERIAL «COLLOST» IN DENTAL SURGERY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 12]

Introduction
Rehabilitation of patients with partial and complete secondary edentia runs with the participation of dentists of different profiles, assumes full satisfaction of both the patient and the doctor [1]. At the present stage it is not the last requirement of the patient's treatment time is. Tooth extraction is the most common surgical intervention dental surgeons carry out. Dental alveole is replaced by mineralized tissue up to 80% in four weeks after tooth extraction [2]. It is the amount of time patient usually wait after last surgery and then goes to prosthetic dentist.
Aim of the study**.** Determination the feasibility of using the collagen material in the surgical phase of the prosthetic treatment with the aim of shortening the duration of treatment by the example of the material «Collost».

Materials and Methods
20 patients participated in the study, aimed at teeth extraction by prosthetic-dentist in connection with chronic apical periodontitis (K.04.5). All patients were male, aged 45-60 years (average age 52.4) with identical values of teeth indices. All patients underwent a professional oral hygiene 7-10 days prior to surgery. Anesthesia was performed with 2% Lidocain. Each patient was removed for 2 teeth in identical segments. One tooth alveole was filled with "Collost powder" and covered with «Collost membrane» (study group, 20 alveoles). The second one was healing under the blood clot (check group, 20 alveoles). Re-examenations carried out on 7, 14 and 28 days.

Results and Discussion
In check group healing process was happening more slowly than in study group. In study group all tooth alveolas were completely healed after 14 days (100%). Those segments of bone had no contraindications for starting prosthetic treatment. In check group there were only 10 (50%) alveolas healed. Concerning complaints there wasn't any in the study group. In the check group about 50% of patients had minor pain complaints.

Osteoplastic materials usually mentioned in literature when speaking about dental implantation and periodontal treatment [3, 4]. There are reports about the possibility of accelerating the regeneration of bone tissue. From our point of view, of great interest is using of collagen osteoplastic materials as a means of shortening the surgical phase of edentia treatment.

Conclusions
Using of «Collost» material create favorable conditions for tissue regeneration after tooth extraction. And prosthetic treatment could be started about 14 days earlier.

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PROPHYLAXIS OF THE CONTACT (GLOVE) DERMATITIS AT DENTISTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 13]

Introduction

Daily stomatologic practice is one of leaders in number of development of undesirable reactions not only at the patient in the dental chair, but also at the dentist, worsening his state of health that in the subsequent is capable considerably to complicate, and sometimes to make impossible further professional activity [1]. In the last decades in connection with continuous use in work of the doctor of gloves abundance of a glove dermatitis considerably increased [2]. This problem fully concerned the practicing dentists of all specialties [3]. The absolute majority of daily used gloves are latex. The risk of allergic reactions to the latex (which is a part of many products of medical appointment) at dentists according to express literature makes 12.7%. Also note that 17% of health workers are sensitized to latex, and at 2% of them it was a cause of illness bronchial asthma [4].

Contact dermatitis from irritation, or an irritative dermatitis makes up to 40% of all facts LA. Both allergic, and not allergic mechanisms share in development of this disease. The latex and chemicals added to a product by production can be the cause of its development. Reaction arises on integuments of hands or any other part of a body after contact with products of latex. The Irritativny dermatitis appears in connection with violations of water balance of skin, in this regard its most reference symptoms are: dryness of integuments, an itch, irritation, a hyperemia, a burning sensation in places of immediate contact of gloves with skin, change of drawing of an integument, crack, rash [5]. Such local manifestations of LA can disappear throughout the short period of time after the termination of contact with latex and use of nutritious creams. The contact and allergic dermatitis makes up to 30% of supervision of LA. It is similar to eczema allergic reaction of delayed (IV) type which usually develops on hands or other parts of a body in 24–48 clocks after the termination of contact with latex products. Not only latex, but also chemicals added to a product can be the cause of such reaction by its production. Local hypostasis, a hyperemia, an enanthesis as an eczema or an urticaria, an itch, cracks, a false skin thickening in places of contact with latex gloves belong to the main clinical signs of a contact and allergic dermatitis above.

Materials and Methods

In researches 22 dentists of Minsk shared. Including 12 volunteers − women, aged from 19 till 49 years which middle age made 29,4 years. Also 10 volunteers − men aged from 19 till 68 years which middle age made 25,5 years shared in research. All surveyed expressed the voluntary informed consent to participation in research. The bioimpedance analysis of a condition of integuments of hands with use of the electronic analyzer «Electronic Skin Analyser» Oriflame was carried out. The following parameters of integuments of hands were estimated: humidity, fat content, a turgor before work in gloves, after work in gloves (latex and nitrile) without use of protective serums, after work in gloves with preventive application of 4 options of serums with various content of hyaluronic acid, a bisabolol and D-panthenol. Any of serums did not contain parabens, mineral oils, synthetic dyes and odorants. Prophylactics used in 10−15 minutes prior to reference processing of hands before reception of patients and therefore, before putting on of gloves. There was enough this interval of time that cream was completely absorbed and began to have positive effect. The period of finding of hands in gloves of objective results, both latex, and nitrile for the purpose of receiving, was standardized and made 3 hours. Researches were conducted by a double blind method: neither the researcher, nor volunteers knew composition of preventive serums.

Results and Discussion

The analysis of the obtained data allowed to reveal that after work in gloves humidity, fat content, a turgor of skin of hands in the common group of the examined dentists worsened for 67%. Use with the preventive purpose of serum No. 1 promoted improvement of an index «fat content» of integuments at 50% of the dentists sharing in research. Use of serum No. 2 promoted improvement of an index "fat content" of integuments in 58.3% of supervision and an index «turgor» − in 41.6%. Use of serum No. 3 allowed indexes «humidity», «fat content» and «turgor» of integuments of hands in 91.7% of supervision, in 8.3% − only fat content of integuments of hands improved. Use of serum No. 4 allowed to improve an indicator «fat content2 of integuments of hands in 83.3% of supervision, indexes «humidity» and «turgor» - in 16.7%.

It is necessary to emphasize that reliable distinctions on the considered indexes characterizing a condition of integuments of hands at the dentists participating in research both when using latex, and at application of nitrile gloves were not revealed. Significant distinctions and on a gender sign were not also revealed.

Conclusions

The presented material gives the grounds to conclude that the condition of integuments of hands of dentists without use of prophylactics considerably worsens on indexes «humidity», «fat content», «turgor» that can be regarded as the prerequisite of development of a contact (glove) dermatitis. The best of the used prophylactics should consider the serum No. 3 promoting simultaneous and to the uniform improvements of all three indexes of integuments.

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BIODEGRADABLE BINARY BLENDS OF POLYLACTIDE AND POLYCAPROLACTONE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 14]

Introduction

Biodegradable aliphatic polyesters like polylactide (PLA) and polycaprolactone (PCL) are widely used in many biomedical applications, including tissue engineering, drug delivery and implant coatings [1,2]. PCL is known for its rubbery characteristic with high elongation at break and for slow biodegradation rate, while PLA is a polymer with high tensile strength and modulus, as well as slower degradation rate. Their properties are complementary, hence they constitute promising blending partners. Polymer blending is a modification technique that allows to combine properties of different polymers or to develop unique features. It is a physical process, thus it does not change the chemical fingerprint of the components, but it can enhance their performance [3].

The aim of this preliminary work was to produce and characterize binary blends of polylactide and polycaprolactone for further use in blood vessels engineering.

Materials and Methods

Two different solvents (Avantor Performance Materials Poland S.A.) were used to prepare initial PLA (IngeoTM 3051D, Nature Works LCC) and PCL (Sigma-Aldrich, Mn = 80 000) solutions – dichloromethane and dimethylformamide in proportion 3:1:

PLA10 (10% w/v PLA),
PCL10 (10% w/v PCL),
PLA15 (15% w/v PLA),
PCL15 (15% w/v PCL).

Samples of 1:0, 1:1, 1:3, 0:1 (PLA:PCL) blends were prepared by solvent blending and casted into films. Digital microscopy, differential scanning calorimetry (DSC), X-ray diffraction (XRD), water contact angle, and tensile tests were used to investigate the structure, morphology and properties of the PLA/PCL binary blends.

Results and Discussion

X-ray diffraction patterns showed sharp crystalline peaks for both pristine polymers (at 16.6°, 19.1° and 22° for PLA, and at 21° and 23.8° for PCL) with lower intensity for lower concentration solutions. Similar peak positions were observed in blend samples, what indicates immiscibility of the two polymers.

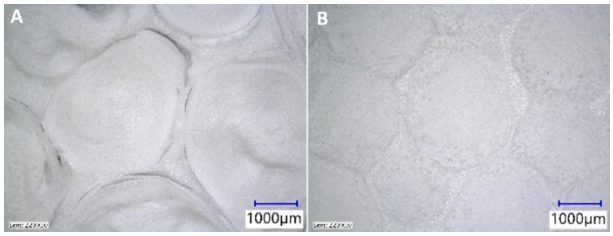

FIG. 1. Digital microscope images of PLA10:PCL15 (A) 1:1 and (B) 1:3.

Also microscopic observations (FIG. 1) and DSC thermograms (FIG. 2) confirmed their immiscibility – in all the blend samples double glass transition temperatures were present. For pristine polymers T_g varied depending on solution concentration: 11.88°C vs. 22.51°C in the case of PLA10 and PLA15, respectively and -59.78°C vs.-56.76°C for PCL10 and PCL15. Moreover, two independent melting peaks corresponding to PCLy (at 62°C) and PLAx (at 169°C) were noted. Blending of PLAx with PCLy resulted in decrease in crystallinity of polylactide component, even up to 31% when higher concentration PCL was added in higher fraction (i.e. PLA10:PCL15 1:3).

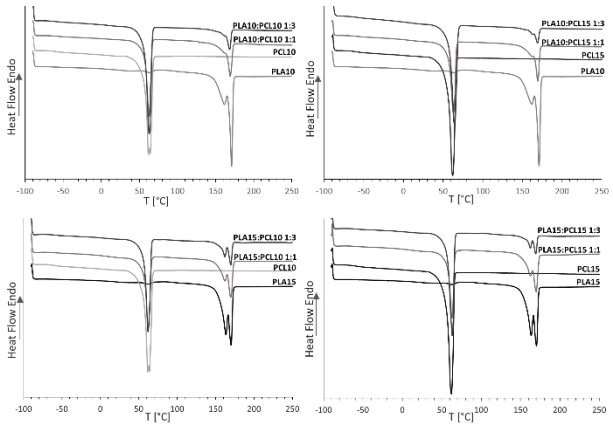

FIG. 2. DSC thermograms of pristine polymers and their blends.

Tensile test revealed that mechanical properties change along with various blend compositions. Pristine PLA is a high strength but brittle material, while PCL is ductile material with high elongation at break. That is why blends with higher PCL content exhibited higher elongation, but lower tensile strength and Young's modulus in comparison to pure PLA.

Conclusions

Blending alters physical properties of the parent polymers, depending on the amount of each polymer. PLA and PCL are immiscible; their blending allows to tailor some tunable properties like crystallinity and mechanical parameters.

Acknowledgments

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BIOACTIVE AND ANTIBACTERIAL COMPOSITE COATINGS FOR MAGNESIUM ALLOYS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 15]

Introduction

Biodegradable composite biomaterials based on polymers offers a wide range of possibilities to introduce various modifications and hence tailor properties of resulting material. It is also possible to use them as e.g. metal coatings. One of the most promising metals nowadays are magnesium and its many alloys, mainly applied for bone and cardiovascular implants [1,2]. In orthopaedic applications, it is important to secure proper bioactivity, which can be achieved for example by addition of various calcium-phosphates, such as hydroxyapatite or tricalcium phosphate [3]. Other essential factor is the bacterial resistance. Bactericidal properties are possible to achieve by incorporation of antibacterial agents, like zinc oxide. Also, Mg itself has the ability to fight bacterial-related infections [4,5].

The aim of this study was to evaluate possibility of using polymer-matrix composite systems modified with tricalcium phosphate and/or zinc oxide for biodegradable coatings of magnesium alloy wires that should protect them from rapid degradation and enhance their biological properties.

Materials and Methods

Magnesium alloy wires (Mg, Leibniz University of Hannover, Institute of Materials Science; d=0.97 mm) with following alloy content: up to 88% Mg, 3% Al, 9% Li and up to 1% of Ca, were etched for 30 s in etching solution (19 g 100% acetic acid, 5 g sodium nitrate (V), 100 ml distilled water), rinsed with dH_2O and dried (20 mins, 70°C). As prepared samples were covered with different polymer solutions by dip-coating method. Coating solutions were prepared by dissolving poly(ε-caprolactone) (PCL, Sigma-Aldrich, Mn=80 000) in dichloromethane (Avantor Performance Materials Poland S.A.) (10% w/v). In case of composite coatings, tricalcium phosphate (TCP) and/or zinc oxide (ZnO) (5% wt.) were dissolved in DCM, homogenised by sonification and then added to polymer solutions to achieve the same final concentration of 10% w/v.

Polycaprolactone and its composite used for coatings were mechanically characterized by tensile test on universal testing machine (Zwick 1435) – tensile strength (Rm, MPa), Young's modulus (E, MPa) and elongation at maximum force ($\varepsilon_{Fmax,}$ %) were calculated. To evaluate potential bioactivity, samples were immersed in simulated body fluid (SBF) in 37°C for 4 weeks, with weekly pH monitoring. Morphology and chemical composition of the precipitates were assessed by SEM with EDS and FTIR analysis.

Results and Discussion

Tensile test revealed that addition of TCP or ZnO alone to polymer matrix does not alter the mechanical properties. However, when two types of particles were incorporated at the same time (PLA/TCP/ZnO system) and in consequence total content of the fillers increased to 10% (in comparison with only 5% when one type was added), there was a significant decrease in tensile strength and even higher reduction of elongation at maximum force observed together with increase in E modulus value.

Immersion in SBF test allows to predict bioactivity of a material. SEM (FIG. 1) with EDS results confirmed presence of calcium-phosphate precipitations on the surface of the samples incubated in SBF for 2 and 4 weeks. Due to degradation of magnesium alloy wires, also some magnesium oxides were visible. The most calcium phosphate precipitated on the surface of the TCP-modified samples (PCL/TCP and PCL/TCP/ZnO coated Mg).

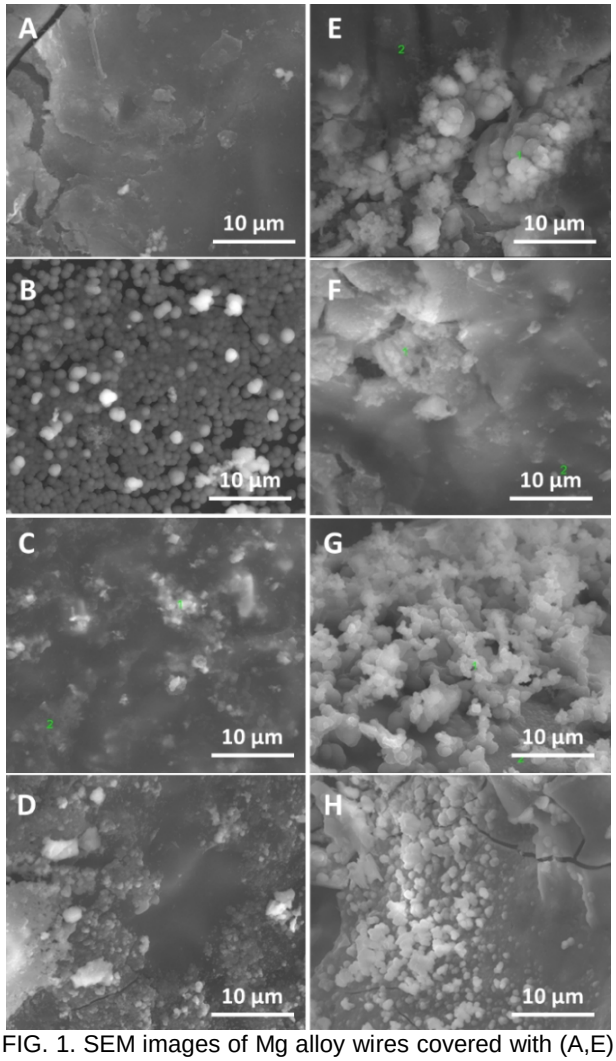

FIG. 1. SEM images of Mg alloy wires covered with (A,E) PCL, (B,F) PCL/TCP, (C,G) PCL/ZnO and (D,H) PCL/TCP/ZnO immersed in SBF for 2 (first column A-D) and 4 (second column E-H) weeks.

Conclusions

The study confirmed that polycaprolactone based composites modified with TCP and ZnO are promising candidates for magnesium alloys coatings with potential bioactivity and sufficient mechanical properties.

Acknowledgments

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THE EVALUATION OF ADHESION BETWEEN ELECTROSPUN COLLAGEN LAYERS AND DIFFERENT TITANIUM SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 16]

Introduction

The local antibiotic treatment of prosthetic joint infection as opposed to the use of systemic antibiotics enjoys the advantage of achieving high antibiotic concentrations, which exceed the minimum inhibitory concentration without increasing the level of systemic toxicity [1]. The aim of our project is a development of nanostructured collagen carrier of antibiotics that will provide a bone/implant bioactive interface, which will enhance the physiological healing process, will be capable of filling bone defects, and will act as a powerful antibacterial agent against microorganisms. The collagen/antibiotic layer is applied to titanium surface directly by electrospinning. The performance of such surface layers is affected by the adhesion between the coating and substrate. This paper presents an assessment of adhesion of pure collagen electropsun layers to differently prepared and treated titanium surfaces.

Materials and Methods

Two kinds of surfaces currently applied to improve the osseointegration of orthopaedic implants were used as substrates for deposition of collagen electrospun layers (FIG. 1), namely titanium plasma sprayed (*S*) and titanium 3D printed (*P*) commercial trabecular surfaces (ProSpon, Ltd., CZ). Prior to electrospinning, all surfaces were chemically treated.

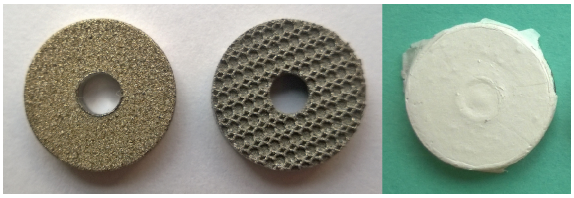

FIG. 1. Representative images of plasma treated and printed titanium samples before and after application of collagen/antibiotic layer (right).

First group of samples (**1**) was degreased by acetone in ultrasound bath (UB) for 10 min. Second group (**2**) was degreased (acetone, 10 min, UB), immediately followed by immersion in PBS (10 min), rinsed with deionized H_2O, dried in a hood. Samples from the third group (**3**) were degreased (acetone, 10 min, UB), etched for 2 min in solution of 5 g Na_3PO_4, 0.9 g NaF, 1.6 g (50wt% HF) supplemented by water up to 100 g, rinsed with H_2O (5 min) followed by fast drying (70°C) and immediately impregnated by diluted collagen/water solution (1/10, w/w) containing N-(3-dimethylamino propyl)-N'-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide (EDC/NHS) (Sigma Aldrich) at a weight ratio of 4:1. After cross-linking in situ, samples were washed in the 0.1 M Na_2HPO_4 (2 × 15 min), followed by rinsing using deionised water (5 min) and dried in a hood. Finally, samples from the fourth group (**4**) were degreased (acetone, 10 min, UB), dried impregnated by diluted collagen/water solution (1/10, w/w) containing EDC/NHS (4/1, w/w) and washed after cross-linking as described above (3). Untreated samples were used as controls (**0**). After these procedures, all samples were immediately coated with collagen by means of electrospinning (1 hour) of collagen solution (8wt% in ethanol/PBS (1/1, w/w) and application of 30 kV, feeding rate 1 ml/hour. The effect of chemical treatment on adhesion of electrospun layers was determined by shaft-loaded blister test (FIG. 2) [2]. The adhesion was quantified based on calculating the maximum bond stress (σ_f) required for layer separation.

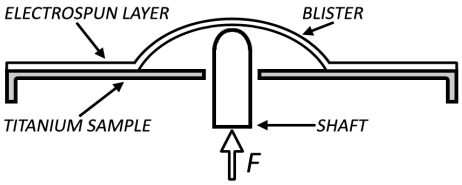

FIG. 2. Scheme of the shaft-loaded blister test, modified from Ref. [2].

Results and Discussion

In terms of bond stress, no differences were found between the plasma-sprayed and 3D printed surfaces (FIG. 3). Various chemical treatments had different effect on adhesion improvement. A very simple method as degreasing or degreasing/PBS immersion can significantly improve the adhesion between collagen electrospun layers and plasma-sprayed substrates. The expected effect of etching was demonstrated in the case of both kinds of substrates. In the case of 3D printed samples, only treatments with impregnation step had a favourable effect on adhesion improvement, while impregnation didn't have any effect in the case of plasma-sprayed substrates.

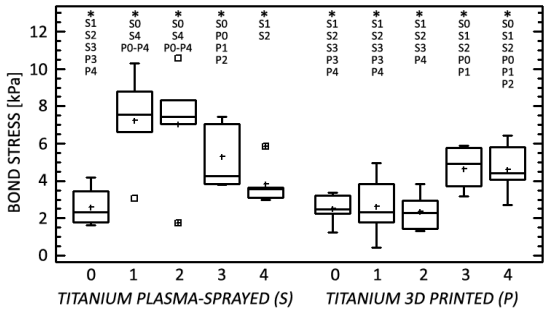

FIG. 3. Bow-plot of bond stress (σ_f) required for separation of collagen/antibiotic layers from differently prepared titanium surfaces. * denotes statistically significant differences (Fisher's LSD, 0.05).

Conclusions

Our results suggest that the adhesion between collagen/antibiotic electrospun layers and titanium plasma-sprayed surfaces as well as titanium 3D printed surfaces can be improved by chemical treatment of titanium surfaces.

Acknowledgments

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«COLLOST» IN THE SURGICAL TREATMENT OF RADICULAR CYSTS IN MAXILLA

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[ENGINEERING OF BIOMATERIALS 138 (2016) 17]

Introduction
Radicular cysts of the jaw bones are from 7 to 12% of all diseases in the maxillofacial region [1]. The outpatient surgical dentistry radicular cysts account for 78-96% of the total number of cysts. Among the operations performed by the dental surgeons as an outpatient procedure, surgery for radicular cysts of the jaws occupies second place after a tooth extraction. Cysts originating from the front upper jaw teeth constitute, according to the literature from 30 to 47% [2].
The main task of the radicular cysts surgical treatment of the jaws is to save the teeth located in the area of the cyst, and the restoration of their functions. Many authors believe that, the main method of surgical treatments cystectomy with simultaneous root resection of the causal tooth without replacement of the bone defect or with bone defect replacement of osteoplastic material. This operation should be performed in the case of existing the tooth root in cyst cavity is not more than 1/3 of its length. A deeper immersion of the root in cyst cavity makes such teeth functionally useless and leads to their early loss.
To prevent the early complications of cystectomy surgeons began to fill bone cavity after removing the cyst with biocomposite materials. Due to the fact that in the case of the standard surgical operation there is a reduction of a blood clot, and this often leads to infection of the bone cavity and following complications.
«Collost» is sterile bioplastic collagen material. It is made of bovine collagen and fully preserving its fibrous structure. «Collost» is a matrix for guided tissue regeneration. «Collost» is collagen absorbable material, it creates transition matrix, which stimulates the body's immune system, activation of granulocytes, macrophages and fibroblasts, the introduction of «Collost» produced new collagen fibers, filling the cavity in the area of implantation, then the implant is gradually resorbed and replaced by autologous tissue. In applying the product «Collost», which acts as a matrix for guided bone regeneration, there is an acceleration of movement of osteoblasts and moving them to a greater distance.
The aim of trial is to study the possibility of applying the material «Collost» at the outpatient dental surgery at carrying out of cystectomy.

Materials and Methods
We used the material «Collost» in the replacement of the jaw bone defects in 5 out patients in 5 outpatient clinic in Minsk, Belarus. These patients were undergone cystectomy surgery of the apex root resection because of radicular cysts in the anterior region of the upper jaw made by a standard protocol.

Results and Discussion
Patients were observed during the postoperative period until the sutures removal for 7 days, 3 months after the surgery. There were carried out the control X-ray examinations to see the bone recovery. Anti-inflammatory therapy during the postoperative period was prescribed as standard.
There were observed no postoperative inflammatory complications in all the patients. During the postoperative period there was absent expressed postoperative soft tissue swelling in patients. The pain in the operated area was preserved no more than 1-2 days; patients did not take analgesics more than 1-2 times during the first 3 days. The increasing of the body temperature has not been observed.
There were no observed cases of «Collost» rejection in the patients, and this is indicating good biocompatibility of the material.

Conclusions
The filling of the jaw bone postoperative defects by collagen absorbable material «Collost» is a method of prevention of postoperative complications in surgical treatment of the upper jaw radicular cysts in the anterior region.

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MULTIREFLEXION X RAY DIFFRACTION METHOD FOR RESIDUAL STRESS INVESTIGATION IN THE Ti-BASED BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 18]

Introduction

Titanium and its alloys are commonly used metallic materials for implants [1,2]. To improve mechanical performance of prosthesis different surface treatment can be applied [3]. The appropriate generated stress (σ) can reduce crack nucleation and propagation and consequently improves fatigue resistance of the implant. Diffraction X-ray methods are commonly used for residual stress (σ) determination [4]. However, standard methods are not advised for the analysis of heterogeneous stress state because the penetration depth (τ) of X-ray radiation varies significantly during measurement. Using proposed in this study MGIXD (multireflexion grazing X-ray diffraction) method it is possible to perform a non-destructive analysis of the heterogeneous stresses for different volumes below the surface of the sample.

Materials and Methods

MGIXD [5,6] was applied for σ determination using laboratory X-ray diffractometer as well as synchrotron radiation for Ti and Ti-alloy (TABLE 1) subjected to different mechanical surface treatments (polishing and grinding).

TABLE 1. Composition of the materials used in presented study (wt.%).

Ti grade 2	Ti: bal. C: 0.010	O: 0.131 N: 0.01	Fe: 0.109 Ni: 0.02	
Ti6Al4V	Ti: bal. C: 0.008	O: 0.20 N: 0.05	Fe:0.25 Al: 6.0	V: 4.0

The laboratory X-ray diffractometer (X'Pert PANalitical) with Cu Kα radiation, was equipped with Göbel mirror to collimate incident beam and the parallel plate collimator in the reflected beam optics. The synchrotron experiment was performed at HASYLAB, DORIS III storage ring, on beamline G3, using soller collimator.The double-crystal germanium monochromator was used. Using synchrotron radiation the τ can be changed for the same incident angle (α) by changing wavelength (λ). Three different λ (1.2527 Å, 1.5419 Å, λ =1.7512 Å) were chosen for this study and the α, for which the penetration depth is the same, were calculated.

Results and Discussion

First the depth-depended σ(τ) profiles were determined from measurements performed on laboratory diffractometer for different α. The obtained results indicate that: stresses close to zero were measured for reference Ti powder, furthermore tensile σ were generated after grinding and compressive σ after polishing. No significant evolution of stresses occurs in the depth-depended stress profile in the case of Ti sample, while the significant gradient of stresses occurs for polished Ti alloy. The results obtained for Ti6Al4V were verified using synchrotron radiation. The example of obtained peak profile is presented in FIG. 1. Strong asymmetry suggests that in fact there are two irradiated regions in the sample with different microstructure. That is why the diffraction peaks were separated into two having different integral widths and position. The broad peak represents 'hard' deformed material in the layer and the narrow one the 'soft' base material.

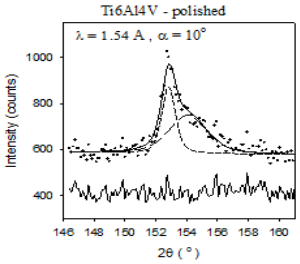

FIG. 1. The example peak profiles for the penetration depth τ = 1.5 μm.

The obtained depth-depended profile of stresses is presented in FIG. 2.

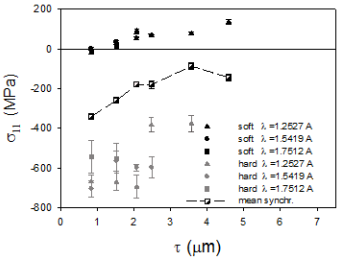

FIG. 2. The depth-dependened profiles of the σ for Ti6Al4V sample. Results after peak separation are plotted as the function of τ.

High compressive stress of about 500-700 MPa has been found in the layer, while in the base material a small tensile stress increases with penetration depth within the range of about 0-120 MPa. A very good agreement of the results obtained using three different wavelengths of synchrotron radiations as well as the classical X-rays (Cu Kα radiation) was found.

Conclusions

The MGIXD is a non-destructive tool which can be successfully used for determination of depth-depended stress distribution in Ti-based biomaterials. Such measurement is possible due to constant penetration depth of X-ray radiation in the studied material. Furthermore the information depth can be easily changed by setting different α. As it was presented in the study the MGIXD method has very important advantages in comparison with other diffraction methods of stress determination especially curtail in the context of biomaterials.

Acknowledgments

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EFFECT OF *IN VITRO* BIODEGRADATION ON THE PROPERTIES OF BNC IN THE ASPECT OF ITS USE AS A MATERIAL FOR THE CARDIAC IMPLANTS PRODUCTION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 19]

Introduction

Bacterial nanocellulose (BNC) is a polysaccharide produced, i.a., by *Gluconacetobacter xylinus* strains. Due to its properties, BNC can be an alternative to materials currently used for production of cardiac implants. Preparation of BNC membrane is relatively inexpensive compared to the cost of obtaining materials from synthetic polymers. In contrast to the commonly used biological tissues, BNC membranes are also easily available. This material meets the requirements of biomaterials: is biocompatible, not mutagenic, toxic or teratogenic. Furthermore, it does not induce immune responses, or the tendency to thrombus formation. However, the level of biodegradability of the material in condition simulating human plasma has not been tested yet.

Materials and Methods

The bacterial nanocellulose, obtained according to using the method described in patents PL 171952 and PL 212003, was supplied by Bowil Biotech Sp. o.o. The susceptibility to degradation by the microorganisms was carried out in the presence of *Staphylococcus aureus* PCM 2054, *Candida albicans* ATCC 10231 and *Aspergillus fumigatus var. fumigatus* ATCC 96918. BNC membranes were stored for six months at 37°C in sterile PBS and SBF fluids in the absence and presence of microorganisms. At selected intervals determined: changes in the wet weight (by gravimetric method), the mechanical properties (by the modified ASTM D882-00 and PN-81/C-89034 norms) and number of microorganisms in SBF fluids.

Results and Discussion

Incubation of the BNC membranes both in sterile simulated human plasma fluids and in the presence of pathogenic microorganisms resulted in a change in the properties of the polymer. The increase in wet weight of the samples was noticeable after 2 months of storage, and after 5 months it achieved 100%. Numbers of cells in microorganism population were increased in the presence of BNC, and maintained on the same level for 6 months. The changes in mechanical properties were the most sensitive method of determining biodegradability. In both the sterile liquids and in the presence of microorganisms, a decrease in tensile strength BNC was already found after one month of incubation. The mechanical properties of the BNC incubated for two months in the presence of *A. fumigatus* could not be measured due to the widespread degradation of the sample.

Conclusions

The method allows for the fastest observe changes biodegradation is to determine the changes in the mechanical properties. Moulds *A. fumigatus* resulted in the strongest biodegradation BNC.

Acknowledgments

This study was supported by the National Centre for Research and Development under Grant PBS II PBS2/A7/16/2013 entitled “Pre-clinical tests of possible applications of the original Polish bionanocellulose (BNC) in regenerative medicine in the aspect of bioimplants in cardiac and vascular surgeries”.

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EFFECT OF *IN VITRO* BIODEGRADATION ON THE STRUCTURE OF BNC IN THE ASPECT OF ITS USE AS A MATERIAL FOR THE CARDIAC IMPLANTS PRODUCTION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 20]

Introduction

Bacterial nanocellulose (BNC), a polysaccharide synthesized by bacteria of the *Gluconacetobacter* genus, is a natural nanomaterial. In comparison with plant cellulose, contaminated with hemicelluloses and lignines, bacterial cellulose is characterized by high purity, high degree of crystallinity and polymerization, and good mechanical properties. BNC is also biocompatible, biofunctional and does not show mutagenicity and teratogenicity. Due to these unique properties, BNC membranes are used for wound dressings, and their potential for the production of cardiac implants is currently under study. Before using BNC as a material for cardiac implants, determination of BNC biodegrabillity in human condition should be examined.

Materials and Methods

The bacterial nanocellulose, obtained according to using the method described in patents PL 171952 and PL 212003, was supplied by Bowil Biotech Sp. o.o. The susceptibility to degradation by the microorganisms was carried out in the presence of *Staphylococcus aureus* PCM 2054, the yeast *Candida albicans* ATCC 10231 and *Aspergillus fumigatus var. fumigatus* ATCC 96918. BNC membranes were stored for six months at 37°C in sterile PBS and SBF fluids, in the absence and presence of microorganisms. Then, changes in the structural properties of the material, determined at selected intervals, were tested by Fourier transform infrared (FTIR) spectrophotometry and X-ray powder diffraction techniques. Changes in the thermal properties of the material were determined by thermogravimetric analysis (TGA), and changes in its surface morphology - by scanning electron microscopy (SEM). The samples were freeze-dried and conditioned prior to analysis for seven days in a P_2O_5.

Results and Discussion

Incubation of the BNC membranes both in sterile simulated human plasma fluids and in the presence of pathogenic microorganisms, resulted in a change in polysaccharide structure. Results obtained after 6-months incubation revealed that the degree of crystallinity of BNC is little changed, and it thermal stability is reduced. Microscopic observations showed loosening the fiber network structure of samples incubated in the sterile buffers and in the presence of microorganisms.

Conclusions

The action of the mold *Aspergillus fumigatus* brings about the strongest changes in the BNC structure.

Acknowledgments

This study was supported by the National Centre for Research and Development under Grant PBS II PBS2/A7/16/2013 entitled “Pre-clinical tests of possible applications of the original Polish bionanocellulose (BNC) in regenerative medicine in the aspect of bioimplants in cardiac and vascular surgeries”.

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SYNTHESIS, CHARACTERIZATION AND APPLICATION OF A NOVEL ZINC(II) ION IMPRINTED POLYMER

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[ENGINEERING OF BIOMATERIALS 138 (2016) 21]

Introduction

Zinc plays an important role in human organism. It is a component of over 300 enzymes. zinc is involved in the synthesis and the breakdown of carbohydrates, lipids, proteins and nucleic acids, and in the metabolism of other trace elements [1]. Both its excess and deficiency can cause a damage to human body systems. Zinc excessive intake may result in a number of adverse health effects including vomiting, fever, nausea, stomach cramps and diarrhea [2]. It is worth mentioning that it may also lead to copper deficiency which is the major consequence of the chronic ingestion of zinc [3].Additionally, studies in rats revealed that a high-zinc diet induces hypocalcaemia and bone resorption [4].

As a result it is really important to control the level of Zn in body fluids. The aim of this work was the synthesis of a zinc (II) ion imprinted polymer which is hydrophilic and can be applied to detect zinc in urine samples by wavelength dispersive X-ray spectrometry.

Materials and Methods

ZnIP (FIG. 1) was prepared by thermal polymerization. ZnSO4·7H2O (1 mmol) was mixed with 1-vinylimidazole (4 mmol) in methanol (20 ml). The mixture was shaken for 1 hour to allow the prepolimerization complex to form. This solution was then mixed with EDMA (15 mmol), HEMA (5 mmol) and AIBN (25 mg). To avoid any side reaction, the oxygen present in the solution must be removed. It is done by bubbling of argon through the mixture for 15 min. The polymerization reaction was performed in an oil bath at 60°C for 24 h. After completion of polymerization, the solid polymer was rinsed with 400 ml methanol followed by 100 ml deionized water, crushed and ground. In order to remove the zinc(II) ions from the polymer matrix, the particles were treated with 17% hydrochloric acid. This process was controlled by the WD XRF analysis of the polymer particles and lasted until no Zn could be detected in the material. The excess of hydrochloric acid was washed by deionized water. Finally the particles were dried in a vacuum oven at 60°C.

Non imprinted polymer (NIP) was also prepared under similar conditions except for adding the template ion.

Results and Discussion

This polymer has been characterized on the basis of FTIR, TGA, TEM and surface area measurement. The imprinted Zn(II) ions were completely removed from the polymer by leaching it with 17% HCl. The optimum pH for the adsorption of Zn(II) on to the polymer was 7. The selective performance of the Zn(II)-IIP polymer was compared to non imprinted polymer (NIP) for the binary mixture Zn^{2+}/Cu^{2+}, Zn^{2+}/Ni^{2+} and Zn^{2+}/Co^{2+}. The relative selectivity of ZnIP was 22.57, 5.440 and 46.17 times greater than that of NIP as compared with the Cu^{2+}, Ni^{2+} and Co^{2+} ions, respectively. At optimal pH value, the maximum static adsorption capacity of ZnIP and NIP was found to be 5.2 mg/g and 0.22 mg/g, respectively. The proposed ZnIP sorbent was applied to determine the zinc ions in urine samples by WD-XRF.

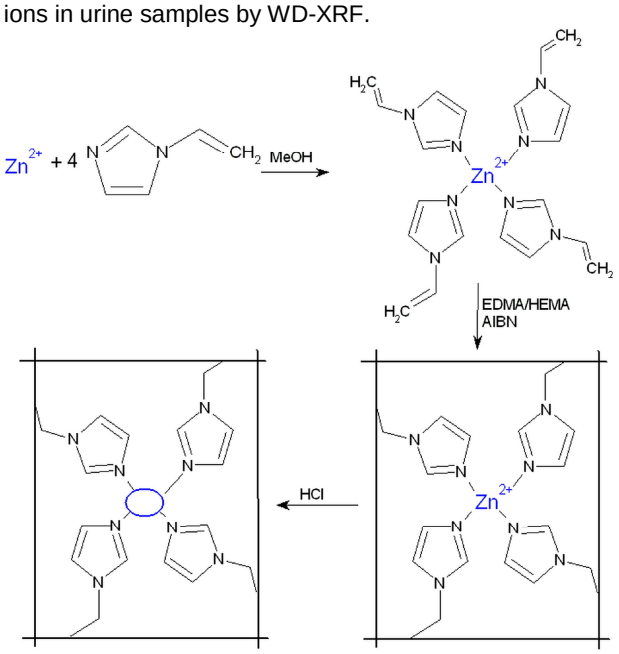

FIG. 1. Schematic illustration of imprinting process for the preparation of zinc (II) imprinted polymer.

Conclusions

The synthesized sorbent exhibits relatively high adsorption capacity and good selectivity towards interfering ions such as: Cu^{2+}, Ni^{2+} and Co^{2+}. Results from the analysis of urine samples have shown that the developed method can be successfully applied for the zinc determination in urine by the WD-XRF method.

It should be noted that the selectivity towards the cobalt(II) ions is the best among the polymers reported in the literature. Although the adsorption capacity of the polymer characterized in this work is lower than some other zinc(II) –ion imprinted polymers its properties seem to be a compromise between sorption capacity and selectivity.

There are research papers which describe molecularly imprinted polymers as drug delivery systems, which can release the therapeutic agent in a controlled way [5-8]. Further analysis of the release of zinc ions from the ZnIP matrix are promising.

Acknowledgments

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EFFECT OF A LOW PH ON HAp/GLUCAN COMPOSITE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 22]

Introduction

The feature of polymer-based composites is volume increase related to swelling in aqueous media. Biomaterial implantation may induce inflammatory response that lead to small environmental changes such as pH. Value of pH may drop to 5.5 in inflamed tissue. Moreover, activated macrophages and osteoclasts can cause even more significant acidification (pH 3.0–3.5) in bone regeneration process [1,2]. These environmental changes may affect the structure and properties of some polymers. Besides, acidic environment may affect also the properties of the ceramic phase due to the dissolution in acids [3]. In this study we investigated the potential influence of microenvironmental changes on ceramic-polymer composite containing hydroxyapatite and β-1,3-glucan. The biomaterial samples were incubated in acidic media during the critical period (5 days) and their physicochemical properties were evaluated.

Materials and Methods

Tested biomaterial was prepared by mixing HAP granules with polysaccharide polymer (83 wt% granules and 17 wt% β-1,3-glucan) [4]. The fabricated material was cut into cylinders 10 mm in length and 9 mm in diameter, dried and sterilized. Individual components of composite, namely glucan samples (control 1) and HAp granules (control 2) were used for comparison. All samples were incubated in Mc Ilvaine citrate/phosphate buffer (pH 7.4, 5.0 and 3.0; 4 mL per well) and incubated in 37ºC for 5 days. The incubation buffer was replaced every 24 h with a fresh buffer. The weight of samples was measured before and during incubation in buffers at defined time points (after 1, 3, 10 min, and 2, 24, 48, 72, 96, 120 h). Ion reactivity of HAp granules used for composite synthesis was examined by analysis of the Ca^{2+} ion concentration in incubation buffers every 24 h for 5 days. Evaluation of physicochemical parameters was performed using microCT, XRD and FTIR analyses, SEM imaging and mercury intrusion technique.

Results and Discussion

The weight and volume of composite samples increased significantly in medium at pH 3 (FIG. 1a-b). Dissolution of ceramics phase in acidic media was confirmed (release of Ca^{2+} ions was presented at FIG. 1f). Pore size remodelling and ceramic phase rearrangement in the composite was shown at FIG. 2.

Conclusions

The elastic composite material (HAp/glucan) swells and undergoes remodeling in acidic media (pH 3.0–5.0). This study enables to predict the optimal quantity of implanted biomaterial and to avoid the overdose effects.

Acknowledgments

The study was developed using the equipment purchased within the agreement No. POPW.01.03.00-06-0109-00. Financial assistance for this research was provided by Medical University of Lublin (grant no. DS.2/14-16).

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FIG. 1.

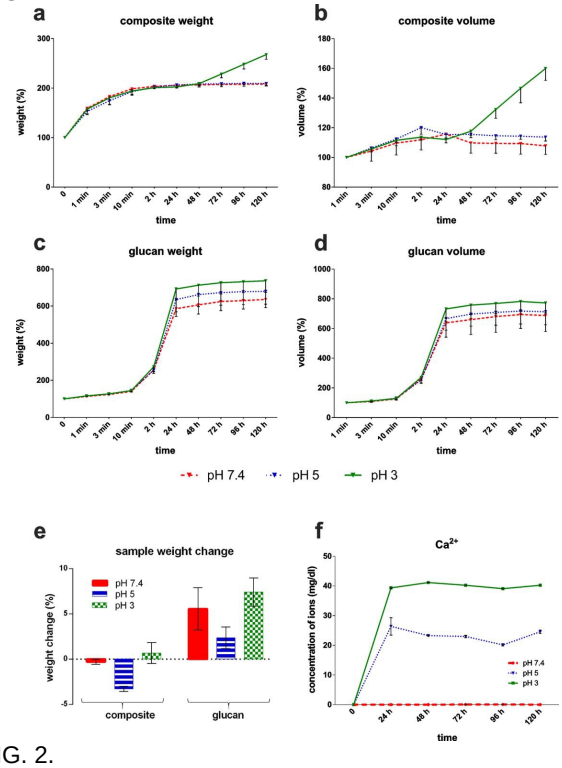
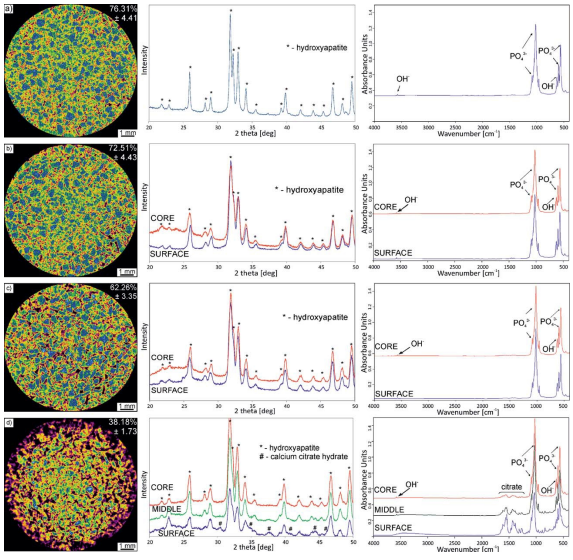


FIG. 2.



NOVEL APPROACH FOR UTILIZATION OF POLY(α-ESTERS) AND MESENCHYMAL STEM CELLS IN VASCULAR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 23]

Introduction
Mesenchymal stem cells (MSCs) are one of the promising stem cells type that may be used in biomedical applications. MSCs are characterized by e.g. huge proliferative and multi-lineage differentiation capacity. Moreover, their low immunogenicity potential makes them suitable for auto- and allo-transplantation with low risk of rejection. Recently, innovative approaches which improve regeneration process, are a wide of interest. They involve utilization of scaffolds composed with MSCs and biomaterials. Thus, the aim of the study was to evaluate the potential of FDA approved poly(α-esters) such as polylactide (PLA) and polycaprolactone (PCL) as a biocompatible and nontoxic substrate for MSCs in *in vitro* culture.

Materials and Methods
In the study we investigated the influence of bioresorbable PLA and PCL polymer-based substrate on morphology, biology and functions of MSCs derived from human umbilical cord Wharton's jelly (hUC-MSCs).
5% polymer solutions were prepared by dissolution of PCL and PLA in glacial acetic acid and dioxane, respectively. Polymers were characterized with scanning calorimetry, X-ray diffractometry and atomic force microscopy techniques. Thin layers of PLA and PCL solutions were poured into culture plate and left for 96h to evaporate the solvents. Prior to cell culture, the PCL and PLA films were rinsed with cell culture medium supplemented with antibiotics.
hUC-MSCs were cultured in DMEM/F12 medium supplemented with 10% FBS, at 37°C with 5% CO_2. The morphology of cells cultured on PLA and PCL surfaces, was examined by several microscopy techniques: light microscopy, fluorescence microscopy and scanning electron microscopy. Cell motility was quantitatively examined via on-live movie recording during cell culture. Time-lapse images were acquired every 10 min up to 30 hrs. Proliferation rate was assessed for every 24 hrs, up to 72 hrs. Cells viability and apoptosis were analyzed by flow cytometry analysis. Furthermore, the influence of PLA and PCL on hUC-MSCs angiogenic differentiation was evaluated through gene expression level by real-time PCR technique.

Results and Discussion
The results demonstrated that both analyzed polymers (PCL and PLA) constitute non-toxic substrate for hUC-MSC growth. Microscopic analysis of hUC-MSCs morphology indicated that presence of PLA and PCL slightly induced stress fibres formation in hUC-MSCs. Data revealed that proliferation rate was partially reduced but analyzed polymers do not affect cell viability. It may be directly associated with properties of culture surfaces e.g. surface topography, crystallinity, wettability and mechanical properties. Moreover, analysis of cells trajectories revealed, that PCL stimulate hUC-MSCs motility by increasing cell speed and total length of cell distance.
Interestingly, the results strongly indicated that the physicochemical properties of culture surfaces play crucial role in hUC-MSCs differentiation process. Our observations suggested that PLA and PCL polymers may preferentially promote spontaneous differentiation of hUC-MSCs towards angiogenic cells. It was confirmed by gene expression analysis of angiogenic cell markers.

Conclusions
In this study we revealed that PLA and PCL constitute suitable substrates for hUC-MSCs culture. Furthermore, we indicated that analyzed polymers may induce spontaneously differentiation of hUC-MSCs into angiogenic cells. We propose novel approach of utilization of scaffolds combined with PLA and PCL polymers and hUC-MSCs in vascular tissue reconstruction.

Acknowledgments
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GRAPHENE-BASED SUBSTRATES INFLUENCE BIOLOGICAL AND FUNCTIONAL PROPERTIES OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 24]

Introduction

Cardiovascular diseases are one of the most frequent causes of death in developed countries [1]. Thus, regeneration of damaged cardiac tissue is leading challenge of contemporary medicine. Recently, significant efforts were placed on stimulation of reparatory mechanisms of injured myocardium, including utilization of mesenchymal stem cells (MSCs) [2]. However, as MSCs cardiomyogenic potential is limited, there are several attempts to increase their therapeutical efficacy, including utilization of biocompatible culture surfaces [3]. Recently, graphene-based biocomposites emerged as promising materials for biomedical applications [4]. However, the possibility of their utilization as surfaces for MSCs culture still requires further investigation.
Thus, the aim of current study was to evaluate the potential of graphene-oxide (GO) and reduced graphene-oxide (rGO) as a culture scaffolds for human MSCs isolated from umbilical cord Wharton's jelly (hUC-MSCs).

Materials and Methods

GO and rGO were prepared from graphite according to the Marcano method. We tested different size and thickness of graphene flakes as well as a type of utilized solvent (aqueous or ethanol), to determine the most effective graphene substrate for hUC-MSCs culture. Next, we investigated the effect of GO and rGO on the biological and functional properties of hUC-MSCs, including morphology, proliferative capacity and migratory activity of the cells. Furthermore, we employed flow cytometry to evaluate the apoptosis rate of cells stained with annexin V and viability dye 7-AAD. Finally, we performed gene expression analyses in order to test the effectiveness of cardiomyogenic differentiation of hUC-MSCs cultured on different graphene-based substrates.

Results and Discussion

Obtained results revealed that graphene-based surfaces constitute non-toxic culture substrates for hUC-MSCs, but their effect depends on the thickness of graphene layer and the level of graphene reduction. Importantly, we observed, that highly reduced rGO flakes affect cell proliferation and survival of hUC-MSCs. Moreover, microscopic analysis of cells demonstrated that graphene-based substrates may stimulate elongation of hUC-MSCs in a flake size-dependent manner. In particular, thicker and larger layers of GO flakes promoted elongated morphology of the cells. Additionally, quantitative analysis of cell trajectories demonstrated that cells cultured on GO prepared in ethanol solvent migrated faster, comparing to the control plates and aqueous GO solution. Importantly, our results shown that GO may enhance hUC-MSCs differentiation toward cardiomyocytes *in vitro*.

Conclusions

Our study provides evidence that graphene-based substrates, particularly GO, constitutes a suitable substrate for hUC-MSCs *in vitro* culture and may enforce functional properties of cells, important for their therapeutical efficacy. However, further studies are required to analyze the impact of several graphene-based materials for SCs culture and their applicability in cardiac regeneration.

Acknowledgments

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STUDYING GLYCOSAMINOGLYCAN DERIVATIVE/PROTEIN INTERACTION - PREREQUISITE FOR THE DESIGN OF FUNCTIONAL BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 25]

Introduction

Numerous biological processes (tissue formation, remodelling and healing) are strongly influenced by the cellular microenvironment [1]. Glycosaminoglycans (GAGs) are important components of the native extracellular matrix (ECM) able to interact with biological mediator proteins [2,3]. They can be chemically functionalized and thereby modified in their interaction profiles [4]. Thus, they can be considered as promising candidates for the design of functional biomaterials to control healing processes in particular in health-compromised patients.

Materials and Methods

GAG derivatives based on hyaluronic acid (HA) and chondroitin sulfate (CS) are characterized in their interaction properties with mediator proteins (MMP-1, MMP-2, TIMP-3, TGF-β1, OPG, and sclerostin) using surface plasmon resonance (SPR; BiacoreT100), receptor binding studies, immunochemical methods and molecular modelling. The biological property profiles of selected GAG derivatives, either alone or being a component of collagen type I-based artificial ECM (aECM) are studied in vitro with cells relevant for healing processes in bone and skin (human mesenchymal stromal cells (hMSC), osteoblasts, osteoclasts, osteocytes, fibroblasts).

Results and Discussion

Biophysical studies show that the interaction profiles between mediator proteins and GAGs are strongly influenced by (i) sulphation degree, (ii) sulphation pattern, and (iii) composition and structure of the carbohydrate backbone. Hyaluronan (HA) derivatives demonstrate typically a higher binding strength in their interaction with biological mediators than chondroitin sulphate for a comparable sulfation degree [5]. Furthermore sulphated GAG derivatives alter the interaction profile of mediator proteins with their cell receptors or solute native interaction partners. FIG. 1 shows this exemplarily for a system comprising the immobilized TGF-receptor II being in interaction with TGF-β1, a GAG derivative, and the TGF-receptor I.

These results are in line with biological effects on cells relevant for wound healing processes. This is valid for solute GAGs as well as those incorporated in collagen-based aECMs. Prominent effects are (i) a tailored degradation behaviour of the native ECM under the influence of MMPs and TIMP-3, (ii) anti-inflammatory, immunomodulatory properties towards macrophages/dendritic cells [6], (iii) enhanced osteogenic differentiation of human mesenchymal stromal cells, (iv) altered differentiation of fibroblasts to myofibroblasts, (v) reduced osteoclast activity [7] and (vi) improved osseointegration of dental implants in minipigs [8].

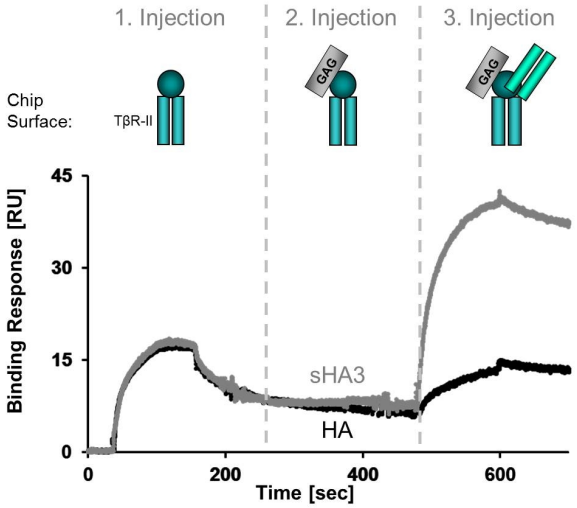

FIG. 1. SPR response curves for the sequential interaction of immobilized TGF-receptor II with solute TGF-β1 (green circle), followed by solute GAG and TGF-receptor I. GAGs: native HA in comparison to sulfated HA (sHA3) with a sulfation degree of 3.

Conclusions

The findings of our consortium Transregio 67 contribute to an improved understanding of structure-function relationships of GAG derivatives in their interaction with mediator proteins and cells. This will enable the design of bioinspired, functional biomaterials to selectively control and promote bone and skin regeneration.

Acknowledgments

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ANTIVIRAL POLYMERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 26]

Introduction
Viral diseases are one of the major causes of mortality in humans since Neolithic period, when people started to form densely populated communities which enabled virus spreading. The general methods of fighting viral diseases include vaccination, treatment with antiviral drugs, isolation and quarantine. They can be, however, applied with only limited success. The number of antiviral drugs is rather small, and the efficiency of the existing ones is decreasing due to the virus mutation. Therefore, there is a need to develop novel antiviral substances which could fight existing and still emerging viral diseases. We have obtained a series of cationic and anionic polymers, both of synthetic and natural origin, which have been shown to inhibit the replication of a series of dangerous viruses such as coronaviruses (CoVs) [1,2], influenza A virus (IAV) [3], and herpes simplex 1 virus (HSV-1) [4].

Materials and Methods
N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC) was obtained by reacting chitosan with glycidyltrimethylammonium chloride (GTMAC) [1,2]. HTCCs with different degrees of substitution (DS) with GTMAC were obtained. N-sulfonated poly(allylamine) (NSPAH) was obtained by sulfonating poly(allylamine hydrochloride) (PAH) with sulfur trioxide - trimethylamine complex (STTC) [3]. NSPAHs with different molecular weights and degrees of substitution with sulfonic groups were obtained. Dextran was cationically modified by substitiution with GTMAC in alkaline conditions [5].

Results and Discussion

Anticoronaviral polymers
Cationically modified chitosans of different DS were found to significantly inhibit human coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1) and murine hepatitis virus (MHV).The mechanistic studies have shown that HTCC inhibits interaction of a virus with its receptor and thus blocks its entry into a cell. The selectivity and the efficiency of the antiviral activity of the polymer depended strongly on its DS.

Polymers inhibiting influenza A virus
NSPAH was found to be nontoxic and well-soluble in water. It strongly inhibited IAV virus replication (FIG. 1). The antiviral activity of NSPAH was proportional to the molecular mass of the chain and the degree of substitution of amino groups with sulfonate groups.

Polymeric inhibitors of herpes simplex virus 1
Cationically-modified dextrans of different molecular weight and degree of substitution with GTMAC were found to have strong activity against HSV-1 virus replication (FIG. 2).

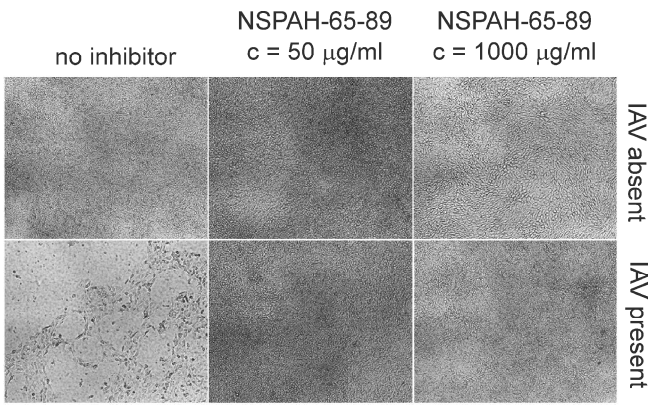

FIG. 1. Optical microscope images present inhibition of IAV in HAE cultures visible as cytopathic effect changes.

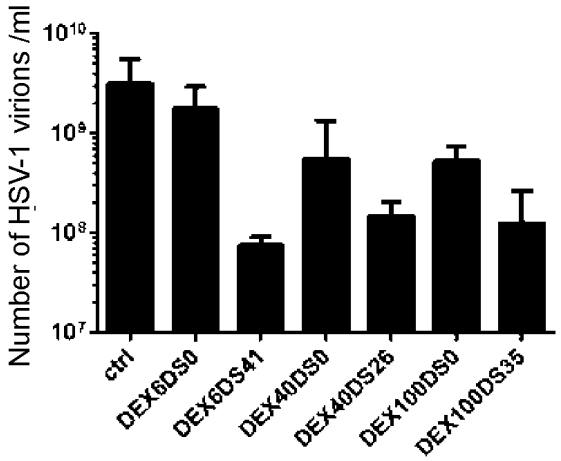

FIG. 2. Anti-HSV-1 activity of dextrans modified with GTMAC. The numbers m and n in DEXmDSn acronyms mean molecular weight of parent dextran in kDa and DS with GTMAC, respectively. Concentration of the polymers was 500 μg/ml.

Conclusions
Novel polymeric inhibitors of HCoV, IAV and HSV-1 viruses have been developed and studied *in vitro*. All of them show strong antiviral effect and can be potentially used as drugs against these dangerous microbes. The mechanisms of their action were determined.

Acknowledgments
The research was supported by the grants of Polish National Science Centre (UMO-2014/13/B/ST5/04510 and UMO-2012/07/E/NZ6/01712), National Centre for Research and Development (Lider/27/55/L-2/10/2011), Foundation for Polish Science (TEAM grant PolyMed, TEAM/2008-2/6 and VENTURES grants Ventures/2009-4/4 and Ventures/2013-11/1), and European Union (grant No: POIG.02.01.00-12-064/08 –"Molecular biotechnology for health").

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CHITOSAN BASED DRUG DELIVERY SYSTEMS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 27]

Abstract
One of the research area in which polymeric materials are intensively explored, deals with the controlled drug delivery systems (DDSs), allowing for drugs distribution directly to the desired site of biological activity. The morphology of polymeric colloidal drug carriers can be described as a construction of a core-shell type. Depending on the chemical or physical bonds providing stability of those systems and the type of interactions between the drug and the polymer, among polymeric DDSs the following morphological structures can be distinguished: micelles, dendrimers, liposomes, niosomes, polymerosomes and micro- and nanocapsules [1]. The main advantages of those systems are the preparation of particles with desired size (diameter from nano to micrometers) during their synthesis / formation and high specific surface area, which can be modified by the appropriate chemical composition of the surface improving the efficiency of a drug delivery.

Chitosan is biopolymer derived from chitin, that is characterised by biodegradability, biocompatibility, mucoadhesion and antimicrobial activity [2,3]. Taking into account the overall advantages of this polymer and the possibility of modification due to the accessible functional groups i.e. hydroxyl and amine, chemically modified chitosan is one of the most promising biomaterials for DDS. In order to obtain micelle structures by self-assemby in aqueous environment several hydrophobically modified chitosan derivatives, such as – stearic acid-modified chitosan [4], palmitic anhydride-modified chitosan [5], linolenic acid-modified chitosan [6], have been synthesized. The micelles prepared by these derivatives in the aqueous medium contain internal hydrophobic moieties as drug reservoir and external hydrophilic chitosan chains as surrounding shell. The above mentioned micellar systems allow encapsulation of hydrophobic antitumor drugs e.g. doxorubicin or paclitaxel due to the compatibility between the hydrophobic core and hydrophobic drug affecting the drug loading and regulate drug release.

Another important group of chitosan based micro- and nanoparticles are those dedicated for gastric infection treatment. The use of chitosan in this specific application is mainly related with the mucoadhesive properties of chitosan resulted from the electrostatic interactions between its positively charged free amine groups and the negatively charged gastric mucins at the acidic stomach pH. Several problems such as high solubility of chitosan under stomach acidic conditions, low retention time and difficulty in crossing the mucus barrier have been observed in those systems [7]. Therefore various crosslinking methods e.g. with glutaraldehyde [8], genipin [9] or sodium triphosphate pentabasic (TPP) solution [10] were investigated in order to minimize these problems.

The nanotechnological production of the polymeric drug carriers, as well as the disadvantages of already developed chitosan based drug delivery systems induce the NANOENCAP project concept on the development and characterization of new dendrimeric micelles polymeric systems, with rigidly defined chemical structure, allowing the encapsulation of several drugs and their controlled release, and thus forming the so-called multidrug therapy systems. To provide the biocompatibility of new polymeric materials the monomer / reactant with proven biocompatibility or naturally occurring in the human body are chosen.

According to the assumptions of the project the amphiphilic character of the proposed multi-functional polymeric drug delivery systems is going to enable the encapsulation of at least two drugs, matching latest trends in the research on DDS models in multi-therapy. As an exemplary multi-drug therapy in this project, the combine therapy of peptic ulcer disease was chosen.

In this work we would like to present the short review of chitosan based drug delivery systems and the concept of the project as well as preliminary studies on new chitosan derivatives and the possibility of synthesis new micellar structures.

Acknowledgments
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ADHESION, GROWTH AND OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS ON POSITIVELY AND NEGATIVELY CHARGED FERROELECTRIC CRYSTAL SURFACES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 28]

Introduction

Cell-biomaterial interaction strongly depends on the physical and chemical properties of the material surface, such as its polarity, wettability, roughness and topography, rigidity and deformability, pH, electrical charge and conductivity (for a review, see [1]). In this study, we focused on the adhesion, growth and osteogenic differentiation of human bone marrow mesenchymal stem cells (hBM-MSC) on uncharged and electrically-charged surfaces with different polarization – positive or negative.

Materials and Methods

The study was carried out on commercially available $LiNbO_3$ substrates (MTI Corporation), namely single crystalline plates, optical grade, dimensions 10 × 10 × 0.5 mm^3, two-sides polished, surface roughness <0.8 nm, (0001) orientation poled perpendicularly to the surface (one surface with the positive charge and the opposite one with the negative charge) and (0100) orientation poled parallel to the surface with zero charge due to the polarization [2]. The samples were sterilized by 70% ethanol, inserted into 24-well cell culture plates (well diameter 15 mm) and seeded with hBM-MSC. Each well contained approx. 10 000 cells/cm^2 and 1 ml of Mesenchymal Stem Cell Medium (ScienCell Research Laboratories). After 6 days, when the cells reached confluence, one half of samples received α-MEM medium supplemented with dexamethasone (10nM), β-glycerolphosphate (20mM) and ascorbic acid (50 μM), and the second half received pure α-MEM. Both media contained 15% of foetal bovine serum, L-glutamine (2mM) and gentamicin (40 μg/ml). The cells on the samples were evaluated for their number, metabolic activity (estimated by conversion of resazurin), type I collagen production (using a Sircol kit), activity of alkaline phosphatase (ALP), calcium deposition (Calcium Colorimetric Assay) and expression of osteogenic markers collagen I, ALP and osteocalcin (using real-time PCR).

Results and Discussion

The number of initially adhering cells on day 1 after seeding, their spreading, shape, and their metabolic activity, production of type I collagen, activity of ALP and Ca deposition in the following days of cultivation (days 6 and 20) were comparable on all three tested surfaces. However, significant differences were found in the expression of mRNA for type I collagen, ALP and osteocalcin, i.e. an early, medium-term and late markers of osteogenic cell differentiation, respectively (FIG. 1). On day 20, the expression of type I collagen was significantly lower in cells on negatively-charged than on non-charged surfaces. Moreover, the expression of ALP and osteocalcin was higher in cells on positively-charged than on negatively-charged surfaces. These differences were generally more pronounced in standard cell culture medium than in osteogenic medium, which could, at least partly, mask the influence of the material surface properties on the cell behaviour. Thus, positively-charged $LiNbO_3$ surfaces seemed to be more suitable for the osteogenic differentiation of bone marrow mesenchymal stem cells than the negatively-charged surfaces.

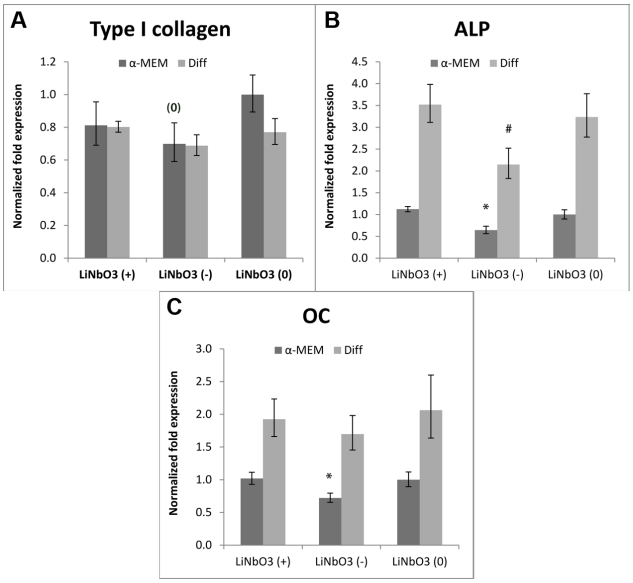

FIG. 1. Gene expression of type I collagen, alkaline phosphatase (ALP) and osteocalcin (OC) in 20 day-old cultures on on positively charged (+), negatively charged (-) and uncharged (0) $LiNbO_3$ surfaces. For the last 14 days, the cells were cultured in standard medium (α-MEM) or osteogenic medium (Diff). Mean ± S.D. from 4 measurements for each experimental group. ANOVA, Student-Newman-Keuls method. Statistical significance: * in comparison with the corresponding samples in α-MEM, # in comparison with the corresponding samples in osteogenic medium, and (0) in comparison with uncharged $LiNbO_3$ sample in α-MEM.

Conclusions

The surface charge of $LiNbO_3$ due to ferroelectric polarization had no significant impact on the adhesion, growth, production of type I collagen and activity of ALP in human bone marrow mesenchymal stem cells. However, the expression of osteogenic markers alkaline phosphatase and osteocalcin was higher in cells on positively-charged than on negatively-charged surfaces.

Acknowledgments

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SUPERPARAMAGNETIC IRON OXIDE NANO-PARTICLE CONTRAST FOR TARGETED MAGNETIC RESONANCE IMAGING OF VASCULAR ENDOTHELIUM INFLAMATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 29]

Introduction

Cardiovascular diseases (CVD), such as atherosclerosis, myocardial infarction and stroke, are the leading cause of morbility and mortality worldwide [1]. Although the medical procedures were considerably improved in the last decades, their general therapeutic results are not satisfactory. That is mostly due to the fact that the therapy is implemented when the disease is already advanced, or quite often even after it has been manifested in cardiovascular events. Thus, there is a need for the sensitive and possibly noninvasive procedures allowing detection of these diseases at the early stage and for novel drugs/therapeutic methods. It is known that all of these diseases begin with the inflammation of the endothelium - the tissue lining the blood vessels [2]. There are studies suggesting that magnetic resonance imaging (MRI) might be useful for early detection of this pathological condition. MRI allows for non-invasive imaging of internal organs and distinguishing healthy from diseased structures at the molecular and cellular level. Unfortunately, it usually requires application of contrast agent. The superparamagnetic iron oxide particles are used for that purpose, but their performance is still not satisfactory. Currently, the main area of research on these systems is focused on the possible improvement of their magnetic properties by decreasing their size to nanoscale region, increasing their biocompatibility and selectivity [3-7].

Materials and Methods

The size and the size distribution of the nanoparticles were characterized by TEM [Tecnai G2 F20 (200 kV) with field emission gun, bright-field, high resolution images. Their hydrodynamic sizes and zeta potentials were measured using dynamic light scattering (DLS, Zeta Sizer Nano ZS). Determination of the magnetic properties was done with a Vibrating Sample Magnetometer, Quantum Design Physical Property Measurement System equipped with a superconducting 9 Tesla magnet. ^{57}Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer. MRI measurements *in vitro* were performed using 9.4 T Bruker BioSpec 94/20 MR imaging system (Bruker, Germany). The content of iron in SPIONs was determined using a classical colorimetric method based on absorbance measurements of the complex of Fe(II) with phenanthroline. The specific interaction of the SPION-VCAM-1 and SPION-P-selectin nanoparticles with the endothelium in the state of early inflammation was studied using the 10 μm- thick cross-section slides of the aorta of diabetic db/db mice at the age of 24 weeks with endothelial dysfunction.

Results and Discussion

The paper presents the results of our studies on development of the superparamagnetic iron oxide nanoparticles (SPIONs) targeted to the areas of vascular endothelium changed in the initial inflammation process, a first step of numerous cardiovascular diseases. The iron oxide nanoparticles (round shape, diameter about 50 nm) coated with cationic derivative of chitosan (CCh) were prepared via co-precipitation of the iron salts in molar ratio Fe(III) : Fe(II) = 2:1 in alkaline deoxygenated aqueous solution of cationicaly modified chitosan. The monoclonal antibodies - anti VCAM-1 and anti P-selectin (0.016 mg per 1 mg of iron), were attached to nanoparticles' surface via tosylation. The permanent attachment of these primary antibodies to the SPIONs surface was confirmed by the immunostaining with IgG-TR (Texas Red) antibodies. Due to the electrostatic stabilization the nanoparticles form a stable colloidal dispersion in aqueous media. The SPION-CCh-anti-VCAM-1 maghemite nanoparticles obtained were superparamagnetic. The *in vitro* studies confirmed the specific interaction of anti-VCAM-1 antibodies bound to the surface of SPIONs with endothelial cells of aorta of db/db mice, known to display endothelial inflammation associated with diabetes. The obtained nanoparticles have also been visualized in aortic arch of the mice with endothelial dysfunction using MRI technique.

Conclusion

Novel, biocompatible, superparamagnetic iron oxide nanoparticles formed stable dispersion in aqueous media and recognizing the area of endothelium changed by early inflammation were obtained and shown to have a potential to serve as a MRI contrast agent.

Acknowledgments

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BIOACTIVE PHOTOCROSS-LINKABLE HYBRID MATERIALS FOR TISSUE ENGINEERING APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 30]

Introduction

Development of the novel biomaterials designed to serve for bone tissue engineering still represents a significant challenge for current regenerative medical research [1]. Scaffolds for tissue reconstruction have been especially extensively studied since there is a need for materials offering new therapeutic opportunities for the repair of bones damaged by trauma, diseases, as well as, due to the aging process [2]. Preparation of the scaffold that would meet all rigorous criteria for maintaining cell growth is very challenging. The scaffold has to serve as a temporary extracellular matrix (ECM), mimic matrix architecture, guide cells, facilitate their growth and provide mechanical support. It is also expected that scaffolds, especially those dedicated for bone reconstruction, will not only mimic the natural extracellular matrix but they will be also bioactive ensuring their interactions with tissue and providing the natural environment and support for bone regeneration [3,4]. Alginate - a linear unbranched polysaccharide is one of the most versatile natural materials known to form hydrogels. This naturally derived polymer is structurally similar to the natural ECM and exhibits low toxicity after purification. Alginate hydrogels are currently being used and explored for a broad range of medical applications including cell encapsulation, drug delivery, as well as, tissue engineering [5]. Gelatin is the basic building block of collagen, a major component of the extracellular matrix and one of the most commonly used protein for creating cellular scaffolds [6]. Silica is a component improving the bioactivity of the polymeric materials.

Materials and Methods

The mechanical properties of the materials developed were studied with a Physica MCR-301 (Anton Paar) rheometer. The mineralization process was controlled using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The cytotoxicity of the materials was controlled using the colorimetric assay XTT with two different cell lines: osteoblasts-like culture (MG-63) and mouse embryonic fibroblasts (MEFs). Statistical significance was calculated using Student's t-test with statistical significance level set at $P<0.05$.

Results and Discussion

This paper presents the results of our studies on physicochemical, mechanical and biological properties of the novel materials fabricated from photocross-linked gelatine/alginate based hydrogels and silica nanoparticles that can serve for bone tissue regeneration. Both gelatin and alginate were functionalized with methacrylate groups allowing their photocrosslinking. Synthetized by Stöber method silica nanoparticles of two sizes were dispersed in three types of polymeric sols namely: the gelatin, alginate and gelatin/alginate, which were subsequently photo-crosslinked and purified by lyophilisation. The swelling ratio, gel fraction and mechanical properties of the materials developed were examined and compared to these determined for plain hydrogel matrices. The mineralization process was conducted in *in vitro* environment in the presence of 1.5 SBF. The materials obtained were exposed to simulated body fluid solution for 7 days and then the morphology and chemical composition of the minerals formed were studied by means of SEM and EDS measurements. The hybrids were synthesised in order to prepare the novel scaffolds, which after swelling can fill up bone defects and provide the conditions essential for the bone tissue regeneration. Considering that application, the biological tests were also performed. The cytocompatibility of the resulted hybrids was evaluated using XTT test against two different cells lines.

It was confirmed that addition of silica nanoparticles to the systems has beneficial effect on the mechanical properties. The storage modulus reached the highest values in the case of gelatin-based hybrids and had the lowest values for alginate-based materials. The *in vitro* cell culture study has shown that the surface of prepared hybrid materials ensures suitable biocompatibility as they can support both MEFs as well as MG-63 mitochondrial activity. Based on XTT test it was demonstrated that addition of SiO_2-nanostructures to the hydrogels does not compromise cytocompatibility of resulted hybrids with respect to plain hydrogels. Finally *in vitro* experiments performed under simulated body fluid (SBF) condition have revealed that due to inclusion of SiO_2 nanostructures into the biopolymeric hydrogel matrices the mineralization is successfully induced. By means of scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) measurements the formation of apatite-like structures in hybrid materials were confirmed. These results clearly illustrate that inclusion of SiO_2 nanoparticles into the hydrogels is beneficial because plain hydrogels (except gelatin) have not induced mineralization under the applied experimental conditions.

Conclusions

Taking into account all data obtained one can conclude that hybrids developed within these studies are promising candidates for bioactive scaffolds in tissue engineering.

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3D ELASTOMERIC SCAFFOLDS FOR CARDIAC REGENERATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 31]

Introduction

Heart failure (HF) is the last stage of cardiovascular diseases, including myocardial infarction (MI). It is one of the most important causes of premature death in the 21st century. MI occurs as a result of a reduction in blood supply to a part of the myocardium. The replacement of diseased myocardium *via* tissue engineering is one of the most promising regenerative therapies to re-establish the blood supply to the affected area[1]. This approach aims to increase the patient´s life expectancy by addressing both the death of cardiomyocyte cells and the malfunction of the muscular heart tissue, in order to avoid heart failure. Here we propose a tissue engineering strategy consisting of three main platforms: (1) Synthesis of novel elastomeric multiblock copolymers. (2) Control of the scaffold production by optimizing electrospinning parameters in order to achieve coiled/curly fiber morphologies that mimic the contractility of the heart tissue; this is expected to provide better biomechanical support for the cells and cardiac muscle regeneration [2,3]. (3) Vary the surface chemistry of the scaffolds in a controlled manner by biologically active compounds.

Materials and Methods

Series of 3D scaffolds utilizing synthesized elastomeric multiblock copolymers of poly(butylene succinate) (PBS) either with dimer linoleic diol (DLA-OH) or dihydroxy poly(isobutylene)(PIB), were produced *via* electrospinning. In order to obtain the coiled/curly fiber morphology, different electrospinning parameters were tested including: voltage, distances between needle and collector, flow rates of solution feeding, copolymer concentrations, solvents mixtures and addition of salts or poly(ethylene glycol) (PEG) to increase the conductivity and viscosity of the solutions. The morphology of the obtained scaffolds was analysed by laser scanning microscopy.

Chemical structures of the synthesized multiblock copolymers were confirmed by ^{1}H NMR and ATR FT-IR spectroscopy. The thermal properties were studied by differential scanning calorimetry (DSC). The wettability of the elastomeric scaffolds was assessed by contact angle measurement and the mechanical properties were measured in tensile tests. Direct cytocompatibility and cell adhesion studies were carried out using murine fibroblasts (L929) on the 3D scaffolds in static conditions.

Results and Discussion

Analysis of chemical structure with ^{1}H NMR and ATR FT-IR spectroscopy confirmed the presence of specific bonds and groups characteristic of the presence of DLA and PIB units in the soft segments of the copolymers. DSC analysis indicated that all of the synthesized copolymers were elastomeric at physiological temperature, which is expected to affect the mechanical properties, degradation profile, and biocompatibility, depending on the amount and nature of the soft segments. Electrospinning process was successfully optimized to produce scaffolds with different morphologies, with an increasing presence of coiled/curly fibers with the addition of PEG or salts to the solutions (FIG. 1).

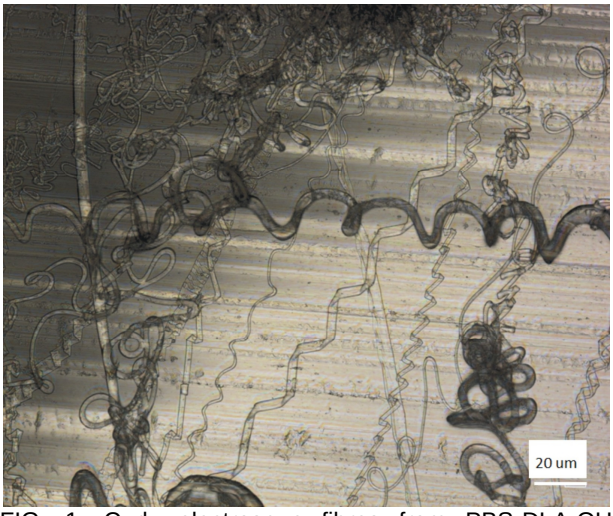

FIG. 1. Curly electrospun fibres from PBS-DLA-OH copolymer containing 50-wt% of soft segments.

The elasticity combined with the curly fiber morphology will result in improved biomechanical match of the scaffold to the heart tissue, improving biointegration. Mechanical tests and cell adhesion studies are being performed to study the effect of fiber morphology and elasticity. Preliminary cytotoxicity experiments indicate that the novel copolymers are non-toxic.

Conclusions

Novel multiblock copolymers, with different hard to soft segment ratios and soft segments building blocks, were successfully synthesized. The ^{1}H NMR, ATR FT-IR spectra and DSC measurements were consistent with the structure and thermal properties expected. The electrospinning process was optimized in order to obtain coiled/curly fiber morphologies. Ongoing studies are characterizing the mechanical and degradation properties, as well as cytocompatibility and cell adhesion. Mechanical and biological properties are expected to be different depending on the compositions and morphologies of the electrospun scaffolds. The future goal of the work is to demonstrate the suitability of the developed materials for cardiac tissue engineering.

Acknowledgments

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GROWTH AND OSTEOGENIC DIFFERENTIATION OF HUMAN OSTEOBLAST-LIKE CELLS ON NANOFIBROUS SCAFFOLDS LOADED WITH DIAMOND NANOPARTICLES: IMPROVEMENT OR IMPAIRMENT?

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[ENGINEERING OF BIOMATERIALS 138 (2016) 32]

Introduction

Nanofibrous scaffolds loaded with diamond nanoparticles (DNPs) are considered as a promising materials for engineering of various types of tissues, including bone tissue. In our earlier studies, nanocrystalline diamond films proved as excellent substrates for the adhesion, growth and osteogenic differentiation of human-bone derived cells, particularly after their doping with boron [1] or termination with oxygen [2]. In the present study, we focused on the cell behaviour of human osteoblast-like Saos-2 and MG 63 cells on PLLA membranes loaded with DNPs prepared by detonation. The obtained results were compared with those in our previous studies, i.e., with the cell behavior on PLGA membranes with DNPs prepared by RF-PACVD method [3,4].

Materials and Methods

Detonation diamonds (NanoAmando, Nanocarbon Research Institute Co., Ltd. Japan) were added on nanofibrous membranes prepared by needle-less electrospinning technique in 6 concentrations ranging from 0.02 to 0.7 g per 100 ml of the polymer solution. After evaporation of the solvent, the concentration of DNPs ranged from 0.44 to 12.28 wt. %. Scaffolds were well characterized by Scanning electron microscopy, IR spectroscopy, Raman spectroscopy, XPS analysis and water drop contact angle.

The scaffolds were seeded by human Saos-2 and MG 63 cells (11 000 cells/cm²) and cultivated at 37°C in a humidified air atmosphere containing 5% of CO_2. We performed the LIVE/DEAD test, ELISA, MTT metabolic test and Real Time PCR to estimate the cell adhesion, viability, growth and metabolic activity of cells on the scaffolds, concentration of specific markers of cell adhesion, osteogenic cell differentiation, cell cycle regulation and apoptosis at the protein as well as at mRNA level. Data were analyzed using ANOVA, Student-Newman-Keuls Method. Statistical significance $p \leq 0.05$.

Results and Discussion

We found that the increasing concentration of DNPs in PLLA nanofibrous scaffolds has rather negative effects on the cell adhesion, viability, metabolic activity (FIG. 1), growth and osteogenic differentiation of MG 63 and Saos-2 cells. In some cases, we observed a slight improvement of the cell behavior on the scaffolds with medium DNP concentrations (1.72 to 3.38 wt. %). These results differ from the results obtained in our previous studies, employing DNPs prepared by radiofrequency PACVD method, In these studies, the cell adhesion and growth on DNP-loaded scaffolds were either unchanged (in MG 63 cells) or even improved (in human bone marrow mesenchymal stem cells) in comparison with the pure polymeric scaffolds [3,4].

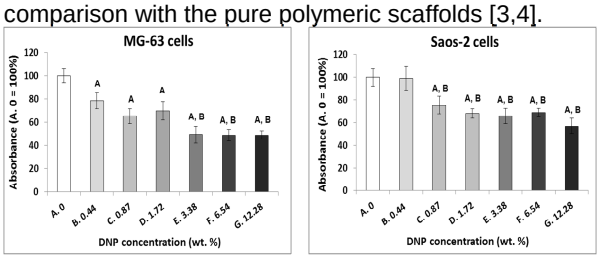

FIG. 1. The mitochondrial activity of human osteoblast-like MG-63 and Saos-2 cells, measured by XTT test on day 3 after seeding on PLLA nanofibrous membranes loaded with 0 to 12.28 wt% of DNPs. Absorbances are given in % of values obtained from pure PLLA membranes (sample A.0).

Consecutively we focused our interest on gene expression of the following markers associated with the regulation of cell cycle and apoptosis: cyclin D, a member of the cyclin protein family that is involved in regulating cell cycle progression; survivin, an inhibitor of caspase activation; Bcl-2 (B-cell lymphoma 2), an important anti-apoptotic protein and oncogene; and KLF6 (Krueppel-like factor 6), a transcription factor involved in growth-related signal transduction, cell proliferation and differentiation, development, apoptosis and angiogenesis, postulated as a tumor suppressor. The expression of cyclin D (FIG. 2), and survivin in Saos-2 cells fell down remarkably with increasing DNP concentration, while the expression of the anti-apoptotic protein Bcl-2 and (KLF6) rose significantly in cells on the scaffolds with lower DNP concentrations (Bcl-2: up to 0.87 wt.%, KLF6: up to 0.44 wt.%), and then decreased. The response obtained in MG 63 cells was weaker.

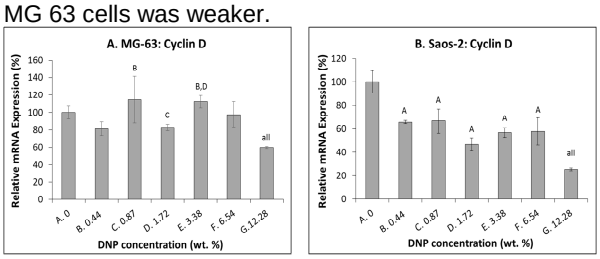

FIG. 2. The expression of mRNA of factors involved in cell cycle progression (Cyclin D) test on day 14 after seeding.

Conclusions

The detonation DNPs might have a direct toxic influence on cells. Thus, the mode of preparation and properties of diamond nanoparticles are important for their biocompatibility and for their applicability in bone tissue engineering.

Acknowledgments

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ELECTROCHEMICAL PREPARATION AND CORROSION RESISTANCE OF DUPLEX THIN FILMS ON TITANIUM ALLOY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 33]

Introduction

In the last decades, the titanium dioxide (TiO2) has been applicated on photovoltaic devices [1], gas and UV light sensor [2,3] and biomaterials [3]. Besides chemical modifications, the nanoscale surface topography of titanium dioxide becomes a crucial factor to be recognized as an attractive and promising for osseointegration for orthopedic and dental implants [4]. Their properties change depending of the fabrication and parameters method. Some chemical and physical modifications of titanium surfaces enhance bioactivity of cells [5].
Electrochemical method is one of methods that are used to produce titanium dioxide layers. It allows the efficient production of highly ordered and closely adjacent nanostructures, which allows an increase in bioactivity and accelerate the process of osseointegration, and also improves the corrosion resistance of the alloy Ti13Nb13Zr [6].

Materials and Methods

Tests were performed on two-phase titanium alloys Ti13Nb13Zr. The electrochemical method was applied to prepare the duplex thin films in two steps. In the first step oxide film was prepared using 1 M orthophosphoric acid (H_3PO_4) for 0.5 h and 1 h at a constant voltage 40 V. In the second step oxide layer was made by the 2 M orthophosphoric acid (H_3PO_4) with an addition of hydrofluoric acid (HF), for 0.5 h at 20 V voltage value. The oxide layers' microstructure, corrosion resistance in Ringer's solution and biological behaviour in vitro were examined.

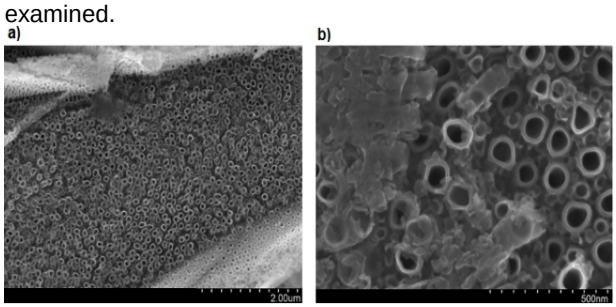

FIG. 1. The microstructure (SEM) of the surface of the sample after electrochemical oxidation at 0,5 h: a) 1 MH_3PO_4, 40 V; b) 1 MH_3PO_4 + 0.3% mas. HF

Results and Discussion

FIG. 1 shows microstructure obtained after electrochemical oxidation. FIG. 2 demonstrates anodic polarization curves for electrochemical unoxidised (FIG. 2a) and oxidised specimens in a solution adjusted to different pH: 3, 5, 7. The specimens oxidised electrochemically showed similar behaviour. For the specimens, two-stage oxidized the appearance of nanotubular oxide layer was dependent on the thickness of the previous oxide layer.
The corrosion tests have been made with potentiodynamic method in Ringer's solution at pH ranged between 3 and 7. The biological tests demonstrated good biocompatibility.

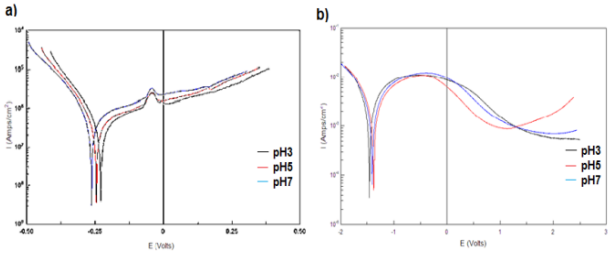

FIG. 2. Polarization curves of the surface: a) unoxidised; b) duplex electrochemical oxidized specimen in different pH.

Conclusions

The obtained results show the formation of duplex layers consisting of two zones- amorphous and nanotubular layers. It has been shown that the duplex films, which improved corrosion resistance of titanium alloy Ti13Nb13Zr even in acidic environment, have been formed.

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FIRST EVER SOLID STATE CROSSLINKING OF HYDROGEL PRECURSORS: OPENING UP UNPRECEDENTED HYDROGEL PROCESSING AVENUES IN THE BIOMEDICAL FIELD

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[Engineering of Biomaterials 138 (2016) 34]

Introduction

Hydrogels are biomaterials often obtained by incorporating a polymerizable double bond in a hydrophilic prepolymer enabling UV-curing. An important requirement for the curing is high mobility of the reactive groups. This is either achieved by dissolving or melting the prepolymers, which is a limiting step for many processing techniques e.g. electrospinning and 3D printing. Radicals initiating the crosslinking can be generated by adding a photo-initiator (PI). Drawbacks of a PI include toxicity, low solubility and mixing problems. To date, UV-curable urethane-based poly(ethylene glycol)(PEG) hydrogels have been described in literature [1,2]. Nonetheless, those materials do not exhibit the unique solid state UV reactivity without PI, as targeted for the novel hydrogel precursors developed in this work [3].

Materials and Methods

Acrylate-terminated, urethane-based PEG(AUP) was prepared by reacting PEG 2000 with isophorone diisocyanate(IPDI) and monoacrylate PEG(336 Da) in a 1:2:2 molar ratio. UV-curing was assessed using rheology and differential photocalorimetry(DPC). 1-Hydroxycyclohexyl-phenyl-ketone (HCPK), 0.2 wt% was used as PI. Cell tests were performed using human foreskin fibroblasts (HFF).

Results and Discussion

In a first part, the precursor was fully characterized using nuclear magnetic resonance spectroscopy, infrared spectroscopy and gel permeation chromatography. The material is solid at room temperature (Tmelt = 37°C) and has a remarkably high water compatibility: even for a AUP content of 90 wt%, a homogeneous solution is obtained. The water content prior to UV curing affects the final characteristics of the hydrogel. These were evaluated using rheology, tensile testing, DPC and swelling tests.
Secondly, and most importantly, the materials show unprecedented photo reactivity. Efficient crosslinking occurs both in the solid state and in absence of a photo-initiator. DPC results (FIG. 1) show that without PI, the maximal polymerization speed is about 50% higher in the solid state compared to the molten state.

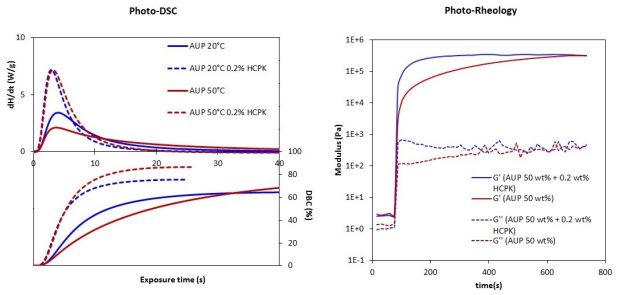

FIG. 1. Left: DPC: UV-curing in PI absence (full line) and presence (dotted line) and in the solid state (20°C, blue) and molten state (50°C, red). The bottom curves show the double bond conversion. Right: Rheology: The red curves are without PI and blue curves with PI.

UV-curing in the presence of water was characterized using rheology (FIG. 1). Adding a PI results in faster UV-curing, while similar final moduli are obtained. This is in line with the DPC results for samples in the molten state.
Some exciting applications of the materials are shown in FIG. 2. A first example is the production of UV-cured hydrogel fibers. As the microfibers are in a solid state post-processing, conventional electrospun hydrogel fibers cannot be UV-cured. However, due to the high solid state reactivity of this material this is possible. Secondly, the material was 3D-printed from melt. The effective UV curing was shown by swelling studies. Very interestingly, the obtained materials were highly flexible.

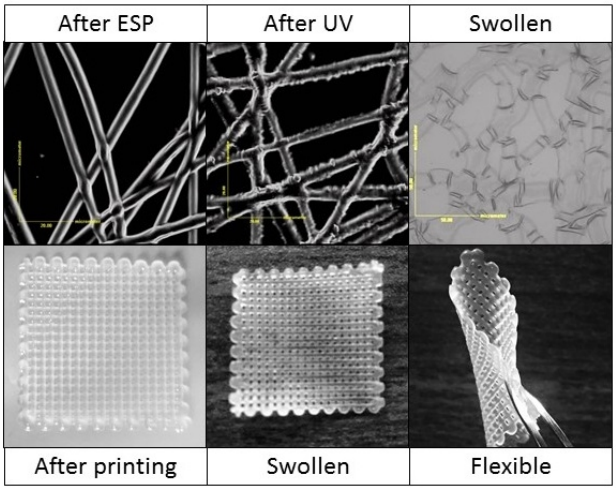

FIG. 2. Electrospun fibers (top), 3D printed scaffold (bottom).

In a final part, indirect and direct cell tests using HFF show good biocompatibility.

Conclusions

We for the first time report on the solid state UV-curing of hydrogel precursors for biomedical applications. The reactivity in the solid state opens up unprecedented possibilities towards material processing, while the absence of a photoinitiator can reduce cytotoxicity and eliminates preparation steps.

Acknowledgments

Authors should acknowledge any person, or funding agency that has made a significant contribution to the work.

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BIOACTIVE COATINGS OBTAINED BY ELECTROPHORETIC DEPOSITION ON Ti15Mo ALLOY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 35]

Introduction

Titanium and its alloys thanks to its unique properties such as good mechanical properties, high corrosion resistance, high levels of hemocompatibility and biocompatibility are attractive materials for medical applications. Materials of this type are often used in implantology such as stents, orthodontic wires, hip, knee replacement or dental implants. Recently, great interest gained the Ti15Mo alloy, it is characterized by high corrosion resistance, biological inertness, low weight and excellent mechanical properties similar to humane bones [1]. Moreover, allergenic and carcinogenic elements such as Ni, Al and V, have been replaced by a biocompatible molybdenum.

Nowadays, one of the methods of enhancing biocompatibility and biofunctionality of the metallic implants is modification of its surface by deposition of biopolymer coatings. Significant interest in electrophoretic deposition (EPD) of polysaccharides such as alginate (ALG), hyaluronate (HYA) or chitosan (CH) is noted [2,3]. Those polysaccharides are natural polymers characterized by high biocompatibility, biofunctionality and non-toxicity. They are widely used in medical applications such as tissue engineering, drug delivery or wound dressing. The great advantage of EPD is that this method does not need high-tech equipment, infrastructure and it is cost-effective.

Materials and Methods

In this work, the EPD method has been developed for the fabrication of biopolymer ALG, HYA and CH films on the Ti15Mo alloy surface. The EPD was performed from electrochemical bath composed with polymer dissolved in aqueous solutions of acid or ethanol. The deposition time and voltage varied in the range 15 s – 1.5 h and 5-100 V, respectively. The microstructure of biopolymer films was studied by SEM and fluorescence microscopy. Formed phases were studied by grazing incidence X-ray diffraction (GIXD). Chemical composition and functional group were examined using EDAX and FTIR methods, respectively.

Results and Discussion

Mechanism and kinetics of EPD of bioactive coatings on the Ti15Mo alloy were discussed. In this work the CH coatings were obtained on Ti15Mo alloy substrate via cathaphoretic deposition and the HYH and ALG coatings were obtained during anaphoretic deposition process. Electrophoretic deposition of biopolymer coatings were conducted for various time-voltage conditions. The analysis shows that with increasing deposition time and voltage increases the mass of the deposited biopolymer. It was found that controlling the deposition conditions it is possible to obtain coatings of variable thickness. The structure of the obtaining coatings is strongly affected by chemical composition of the electrochemical bath and parameters of the deposition process. Using acetic acid solution as a one of the deposition bath component increased the hydrogen evolution and caused the porous coatings creation. Whereas coatings deposited from citric acid bath are smooth and homogenous. Utilization of acetic acid in electrochemical bath greatly increasing the deposition yield of CH coatings. Also higher concentration of ethanol in electrochemical bath increase deposition mas of HYH on Ti15Mo surface.

X-ray phase analysis confirmed the presence and the amorphous nature of the deposited biopolymer coatings on the Ti15Mo alloy. ATR-FTIR analysis allowed to determine the characteristic functional groups of the deposited biopolymer coatings.

Conclusions

Based on the results it can be stated that electrophoretic deposition is appropriate and optimal technology for biopolymer coatings formation. Degree of coatings porosity and thickness can be diversified by the variation of deposition conditions and chemical composition of the bath.

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THE INFLUENCE OF MODIFICATION OF METALLIC MEDICAL IMPLANTS COATED MULTI-DOPED CARBON LAYERS (DLC, DLC-Si AND DLC–Si/Ag) ON CHANGES ON THE IMPLANTS SURFACE AS A RESULT OF IMPLANT - BONE CONTACT CONSIDERING ORTHOPEDIC SCREWS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 36]

Introduction

There is an increasing demand for metallic implants, new technology of them manufacturing and improvement their mechanical and tribological properties. In USA, the annual increase of total hip and knee arthroplasty declared for 2010 was estimated on the level of 4.3% [1], while in England and Wales between 2008 and 2010, the number of such treatment changed from 109825 to 166000 [2]. One of the most important aspects is the interaction of biomaterials with the tissue through its surface. The possible path of implant development is connected with the use of diamond-like carbon coatings (DLC). DLC coatings determine a diffusive barrier [3] preventing tissue near implantation site from penetration by metal ions, which can lead to their deposition (in spleen, liver, blood, etc.), allergies or irritation effect [4]. To improve the biological properties, with the invariance of the mechanical and physicochemical properties, new ways of metallic implants surface modification are searched.

Materials and Methods

Presented work concerns surface properties of carbon layers synthesized on metal substrates with use the RF PACVD method. The process of the synthesis comprised two stages. The first stage was heating and making uniform the temperature on the coated elements (at the pressure 20 Pa and negative potential electrode polarization -1200 V). The second stage was aimed at the production of the coating (about 35 Pa and polarization of the electrode -1000 V). Investigated layers were deposited from methane plasma onto stainless steel 316LMV substrates. For the investigations samples were used orthopaedic screws and 16 mm in diameter, 6 mm in thickness samples made of stainless steel [5].

Results and Discussion

DLC, DLC-Si and DLC-Si/Ag layers presented very good mechanical properties e.g. good adhesion to the substrate surface. The layers are characterized by the homogeneity and uniformity. The AFM investigations indicate the structure being typical of nanocrystalline layers.

To define the mechanical properties to the surface there was a device NanoIntender G200 applied. The nano-hardness of the layer surface established on the level 13 GPa, and Young Modulus 230 GPa and the critical adhesion force was equal to 50 mN.

Manufactured DLC layers presented thickness about 200 nm.

To provide the chemical composition of the coatings X-ray electron spectroscopy was used.

Investigations of the orthopaedic screws were performed on a UMT-2 Bruker. The test consisted in realization of drilling process. During the test, the screws were tightening and loosening in beef bone.

Firstly the bones were carefully cleaned from soft tissue. The bones were kept in an airtight container at a temperature approx. 6°C. Before the test, bones were drilled. The holes have 8 mm diameter. The rotational speed of machine was 12 rev/min. During the test constant axial force of 40 N was applied, increasing load on screws. The tests were conducted on the screws with DLC and DLC-Si modification and unmodified ones [6-8].

Investigations of the orthopaedic screws were performed on a UMT-2 Bruker. The test consisted in realization of drilling process. During the test, the screws were tightening and loosening in beef bone. Firstly the bones were carefully cleaned from soft tissue. The bones were kept in an airtight container at a temperature approx. 6°C. Before the test, bones were drilled. The holes have 8 mm diameter. The rotational speed of machine was 12 rev/min. During the test constant axial force of 40 N was applied, increasing load on screws. The tests were conducted on the screws with DLC, DLC-Si and DLC/Si-Ag modification and unmodified ones [6-8].

Conclusions

The obtained results showed that the DLC and DLC doped Si and Ag layers manufactured by RF PACVD method have very good mechanical and tribological properties. On the surface of modified orthopaedic screws, after cycles of drilling, is formed tribofilm made of mineral components. The tribofilm is homogenous and uniformity.

After testing with required parameters, the DLC, DLC-Si and DLC-Si/Ag coatings were not broken.

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DESIGNING AND PREPARING BY FDM 3D PRINTING OF POLYMERIC SCAFFOLDS WITH POTENTIAL APPLICATION IN TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 37]

Introduction

The aim of this research is to develop a procedure to prepare the biodegradable polymeric scaffolds using 3D printing by fused deposition modeling (FDM).

Mechanical damages of the bone and cartilage tissue within joints are a common result of sport and communication accidents. This type of defects usually causes a pain and limitation of a movement range. The basic problem of the therapeutic procedure is a limited ability of the cartilage tissue to regenerate [1]. One of the most effective methods of supporting of this process is to apply the polymeric scaffolds with shape, dimension, infill and material adequate to the type of the damage.

3D printing by fused deposition modeling is one of the most interesting method of the polymers processing [2,3]. Thermoplastic material in form of a filament is melted and casted layer by layer to produce three dimensional object. The structure of this product could be precisely described by designed computer model.

Materials and Methods

In the presented research an application of low-cost FDM 3D printer and freeware software is evaluated. RepRapPro Tricolour Mendel (RepRapPro, United Kingdom) is a commercially available device designed by open-source project RepRap. Blender, Sli3er and Pronterface are the GNU GPL software used to design the structure of the object and to prepare the print. Poly(L-lactide) (PLLA) and poly(L-lactide-co-trimethylene carbonate) 15/85 (PLLATMC) were purchased from BioMatPol, Poland. The printed polymeric scaffolds were analyzed using Keyence VHX-900F digital microscope.

Results and Discussion

The structure of the scaffolds including shape, dimension and infill was designed using Blender software. Cylinders with 10.0 mm height and 10.0 mm diameter were prepared with three different infill patterns resulting in 60% porosity. The rectilinear patterns with 0.3 mm bar diameter and 0.3 mm layer height include orientation 0°/90° (type A), 0°/60°/120° (type B) and 0°/45°/135°/90° (type C). Designed models were saved as an .stl files.

The filament with 1.75 mm diameter was prepared using a screw extruder. PLLA/PLLATMC 80:20 blend was selected as a material appropriate for bone tissue regeneration.

The optimization of the printing process was conducted using Sli3er software to get a required quality of the printed structures. The most important parameters include a nozzle temperature (200°C) and the speed of the printing movements (10 mm/s). Prepared .gcode file was uploaded to Pronterface software used for the printer controlling.

Microscopic images of the printed polymeric scaffolds are presented in FIG. 1.

Conclusions

The developed procedure enables to design and prepare the polymeric scaffolds with required structure using low-cost 3D printer and freeware software. The shape, dimension and infill are precisely specified by 3D model design. The selection of biodegradable thermoplastic polymer for filament preparing depends on the potential application of the scaffold in tissue engineering.

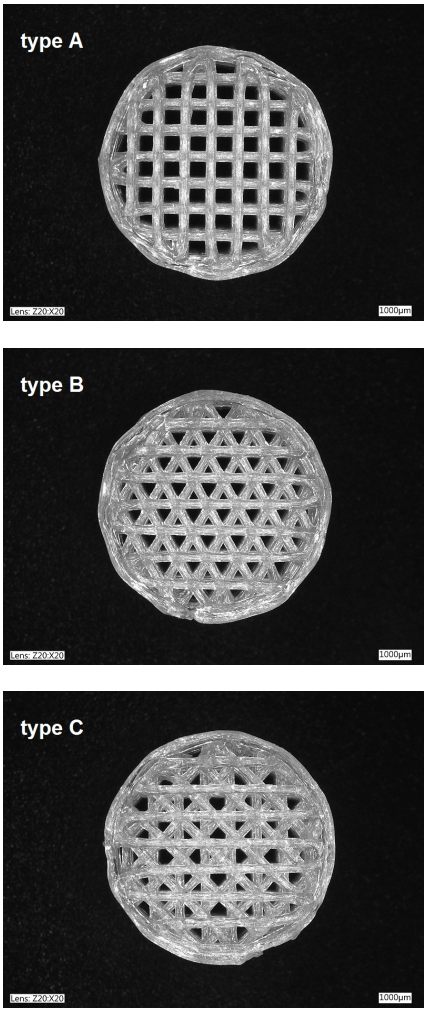

FIG. 1. Microscopic images of the polymeric scaffolds.

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SYNTHESIS AND CHARACTERIZATION OF AMPHIPHILIC CHITOSAN DERIVATIVES AS NANO/MICROSTRUCTURES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 38]

Introduction

Among the most popular drug delivery systems (DDSs), dendrimers and polymeric micelles have gained attention due to certain structural advantages and features that they offer. Dendrimers are characterized by a precise molecular weight, high density of surface functionalities and well-defined structure that allow a high payload and provide toxicity control and release properties [1]. While, polymeric micelles based on amphiphilic polymers with self-assembly properties have the unique core-shell structure, a micro or nanoscale size, and thermodynamic stability [2].

One of the most abundant biomaterials - chitosan can also be an amphiphilic, by a hydrophobic modification, and create the proper derivatives for the above purpose. Moreover, most chitosan derivatives due to unique properties as low toxicity, excellent biocompatibility and biodegradation are suitable as a drug delivery vehicle [3].

The structural combination of those two systems i.e. polymeric micelle and dendrimer, named as dendrimeric micelle (DM), might allow creating a new multifunctional and multidrug delivery system.

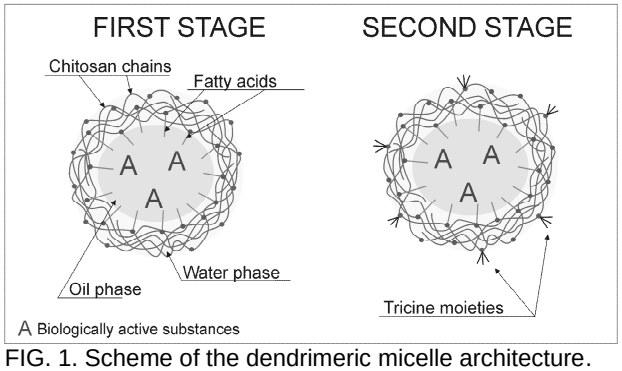

FIG. 1. Scheme of the dendrimeric micelle architecture.

In the study, amphiphilic chitosan derivatives were synthesized, characterized and tested for micelle formation abilities for the first step of a designed DM construction.

Materials and Methods

Series of amphiphilic chitosan derivatives were synthesized using carbodiimide EDC or EDC/NHS catalysis. The modifications were proceeded by introducing long fatty acid chains as hydrophobic moieties and/or tricine (*N*-(tri(hydroxymethyl)methyl)-glycine) as hydrophilic moieties.

Application of different reaction conditions allowed to obtain various *O*- or *N*- and *O*-,*N*- substituted derivatives.

The structures of the obtained materials were confirmed by ^{1}H NMR and ATR FT-IR spectroscopy.

Micelles formed from the derivatives were obtained by the O/W emulsification technique using dichloromethane (DCM) and 1wt% acetic acid (and ethylene glycol (EG)) as oil and water phases, respectively. The influence of the derivative concentration, DCM, and EG ratio on the micelle properties was investigated. The characterization of the micelles was performed using the dynamic light scattering technique by Zetasizer Nano-ZS (Malvern) apparatus determining the hydrodynamic diameter of the micelles.

Results and Discussion

The chemical structures of synthesized polymers were determined by ^{1}H NMR and ATR FT-IR spectroscopy. The successful incorporation of the fatty acid groups and/or tricine moieties onto the chitosan backbone was confirmed by ^{1}H NMR assay. The FT-IR spectra of the derivatives showed characteristic absorption bands for *N*- or *O*- and both *N*-, *O*- substitution depending on the reaction condition used.

The amphiphilic character of derivatives facilitated the micelles formation. By changing the solvents ratio as well as the derivative concentration, different micelles diameters were obtained, ranging from micro (ca. 55 µm) to nano (ca. 42 nm).

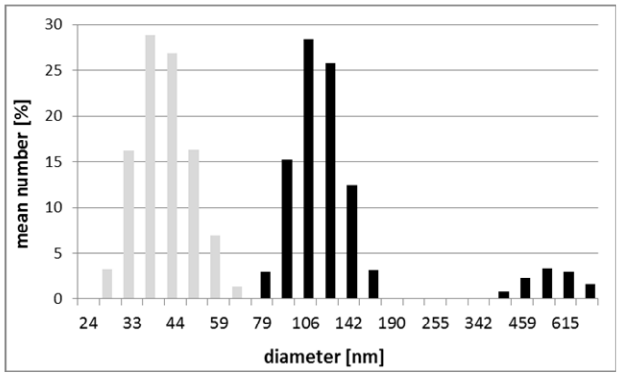

FIG. 2. Dynamic Light Scattering results of micelles prepared from 1g/L (black) and 0.8g/L (grey) chitosan derivative concentration in 1wt% of AA and 3v% of DCM.

Conclusions

As a part of the ongoing multifunctional polymeric drug delivery systems project (NanoEnCap), we reported herein the synthesis of amphiphically modified chitosan molecules with fatty acid chains as hydrophobic moieties and/or tricine group as hydrophilic ones. Providing the different O/W emulsification conditions, the self-assembly process was controllable and led to micelles with tunable sizes, which are suitable for further development of the dendrimeric micelle construction.

Acknowledgments

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MICRO-CT ANALYSIS OF BONDING OF CONVENTIONAL AND GLASS FIBRE COMPOSITE FILLINGS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 39]

Introduction

Recently, composite materials are reconstruction materials which are most frequently selected by the dentists and patients. Owing to micro leakage and inner cracks of the material, the efforts are continued in order to improve their properties among others to increase their mechanical resistance [1]. The use of composite with glass fibres and conventional composite enables the biomimetic reconstruction of tooth structure. EverX Posterior based on glass fibres in vivo tests carried out by Garoushi at al. [1,2] indicate to good clinical properties of this material and in vitro tests demonstrated resistance of this material to the impact of variable loads [3]. Except of chemical adhesion, the fibres maximize the bonding in order to ensure proper bonding with each composite applied thereon and with tooth tissues [1,3-5].

With the advent of advanced structures imaging techniques, non- destructive tests by means of CT technique are an excellent tool used for materials quality analysis. CT is used by authors for monitoring of polymerization shrinkage and micro leakages in teeth tissues and in fillings. By means of CT method it is also possible to precisely determine the location of porosities and other discontinuities.

The purpose of the study is to evaluate the bonding of composite material reinforced with Glass fibre with conventional composite and tooth tissues by means of computer micro-tomography. 2D and 3D analysis has been carried out for quality of filling and adhesion on the interface between two different composites including tooth tissues.

Materials and Methods

Third molars without carries extracted due to orthodontic reasons have been used in the test. Composite biomaterial reinforced with glass fibre - everX Posterior manufactured by GC in the form of single layer and Filtek Z250, 3M ESPE conventional composite were applied in the form of layer 2-3 mm.

Micro – CT tests have been performed by means of SkyScan 1174 computer micro-tomography (Bruker, Belgium). Software: NRecon ver. 1.6.10.4 for image reconstruction, 2D DataViewer ver. 1.5.2.4 for analysis and 3D CTVox ver. 3.1.2 for 3D analysis were applied.

Results and Discussion

FIG. 1 illustrates reconstructed 2D images of the tooth in XYZ axes. No porosity has been detected in the both fillings, the structure seems to be uniform. White line represents the contour of visible interface separating two fillings.

So called “structure recess” has been specially completed in FIG. 2 in order to disclose the quality of internal structure. Also in this case, the border between the both fillings is visible. No porosity or micro leakage has been observed.

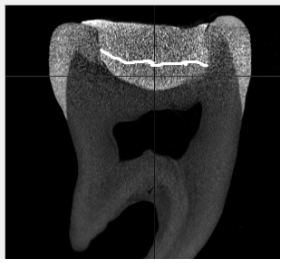

FIG. 1. Reconstructed 2D sections in XYZ axes: marked contour of border between the both fillings; computer micro-tomography.

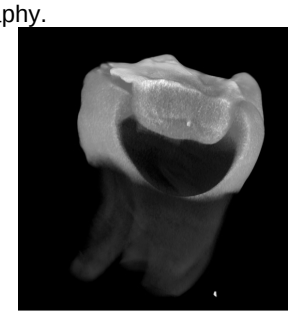

FIG. 2. 3D view of third molar structure: visualization of the central part of tooth with visible fillings; computer micro-tomography.

Combination of materials with various structure is the key factor in layer technique which determines the durability of losses filling. Covering of fibres protruding from everX Posterior filling with the second material is important owing to lack of techniques of shortening of removal of fibres protruding from material.

Conclusions

The tests carried out by means of computer micro-tomography are an alternative for X-Ray examinations as a non-destructive ensuring 2D and 3D visualization. Analyzed structure of the tooth and composite materials confirms high quality of the filling and the lack of micro leakages between the filling and tooth tissues. The filling performed by means of layer technique is free of porosities. The presence of proper bonding has been confirmed on interface between the both applied materials: everX Posterior and conventional composite.

Acknowledgments

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THE SUSTAINABLE RELEASE OF VANCOMYCIN FROM MICRO- AND NANOSTRUCTURED COLLAGEN LAYERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 40]

Introduction

The infection of implanted endoprostheses represents a serious problem as far as orthopaedic and trauma surgery are concerned. One of the ways in which to increase the efficacy of the therapy is to use a local antibiotic delivery system [1]. Local delivery of antibiotics maximizes target tissue concentration, and minimizes systemic toxicity risks. Technology and conditions during such carrier's preparation are very important aspects as they can greatly affect the final release profile of antibiotics or their transformation to microbiologically inactive products. The aim of the study was to compare biodegradable composite layers prepared by different techniques. They should release active form of Vancomycin in a sustained and controlled manner effectively for 3 weeks at concentrations exceeding minimum inhibitory concentration for vancomycin-resistant *Staphylococcus aureus* (VRSA) (>16 mg/L) without initial burst releasing.

Materials and Methods

Micro- or nanostructured layers were prepared based on collagen (type I, VUP Medical, CZ), 0, 5 or 15 wt% of hydroxyapatite nanoparticles (avg. 150nm, Sigma Aldrich) and VANCO (Vancomycin hydrochloride, Mylan S.A.S, France) in amount of 10 wt% of total weight of collagen (COL) with hydroxyapatite (HA). Micro-structured layers were prepared by means of lyophilisation of COL/HA/VANCO dispersion [1]. Nano-structured layers were prepared employing the electrospinning (4SPIN, Contipro, CZ) of 8wt% collagen solution with dispersed HA particles. VANCO was applied by two different procedures, i.e. directly to COL solution before electrospinning or subsequent impregnation of electrospun COL/HA cross-linked layers, respectively. The stability of all collagen layers were enhanced by cross-linking with EDC/NHS (Sigma Aldrich) [1]. The solid phase extraction method and HPLC analysis [2] (Agilent 1200, diode array detectoreDAD, Agilent Tech.) were used to characterize the *in vitro* release rates of VANCO and its crystalline degradation antibiotically inactive products over a 21-day period (PBS, 37°C). The antimicrobial effects of the layers were determined using agar diffusion testing against VRSA isolates.

Results and Discussion

The structure of micro and nanostructured layers is illustrated at FIG. 1. The maximum concentration of the released active form of vancomycin (approx. 700 mg/l after 3 hours, 150 mg/l 21st day) was assessed by means of the vancomycin impregnation of cross-linked electrospun layers.

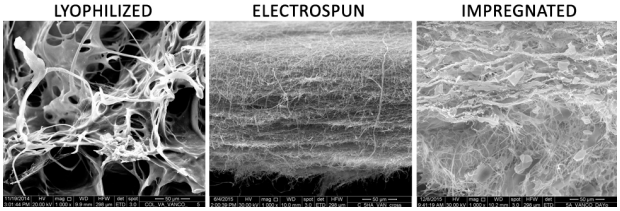

FIG. 1. Representative SEM images of COL/5%HA/ VANCO layers prepared by different techniques (x1000).

The lowest concentration was determined for those layers electrospun directly from a COL solution with VANCO (see FIG. 2). Modification using hydroxyapatite exerts no strong effect on vancomycin evolution.

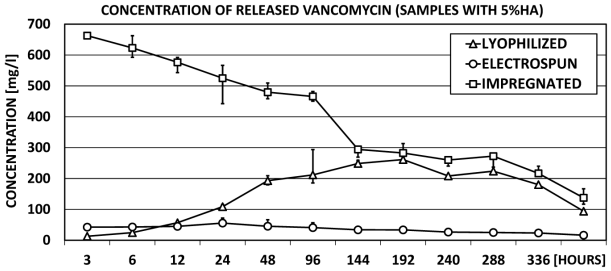

FIG. 2. An example of the concentration of released active form of vancomycin from layers with 5%HA (median, interquartile range).

Agar diffusion tests (FIG. 3) revealed that the electrospun impregnated layers exhibited the highest activity. All the tested layers showed release of an active form of VANCO release at concentration higher than MIC.

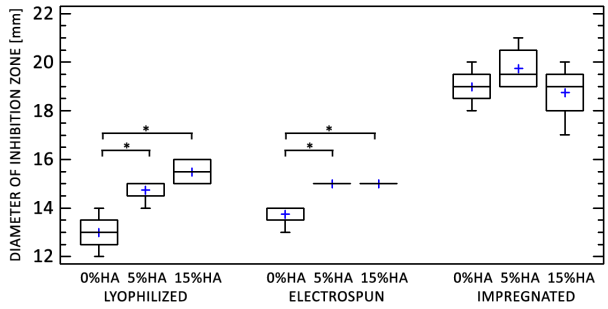

FIG. 3. Diameter of the inhibition zone against *S. aureus* as function of different HA concentrations and different preparation techniques (Mann-Whitney, 0.05).

The higher specific surface of nanostructured layers probably plays a negative role in the preparation process due to the higher rate of VANCO elution to the cross-linking solution. This may be overcome via the subsequent impregnation of the cross-linked layers.

Conclusions

Our results suggest that the local application of high-dose vancomycin via drug delivery carriers provides a safe therapeutic osteomyelitis treatment method that prevents the development of bacterial resistance.

Acknowledgments

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BIORESORBABLE SELF-EXPANDED VASCULAR STENTS - THE PREELIMINARY RESULTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 41]

Introduction

Because of frequently encountered late complications related to implantation even the latest generation of vascular stents, optimal treatment of coronary heart and peripheral artery disease, including acute coronary syndromes entails the need to search for new solutions. Fully biodegradable scaffolds represent the most ambitious emerging stent technology. The rationale is to provide a stable vascular stents in the short term, thus minimising restenosis due to vascular recoil, constrictive remodelling and loose intimal dissection flaps [1,2]. Thermoplastic poly-L-lactide (PLLA) is the most frequently used in the manufacturing of this type of scaffolds, which are formed similarly to the typical metal stents, via laser cutting to the final stent shapes previously fabricated by extrusion of mini-tubes. Currently being clinically used balloon-expanded stents such as ABSORB or Igaki – Tamai stent are received this way. However most interesting looks the possibility to form this type of tools with the help of new stunning technology - microinjection moulding, which should greatly cheapening of their production and overcomes stents shape design restrictions related to the used so far manufacturing method with laser punching. Injection moulding allows to remove troublesome problems in giving the stent's final permanent shape too. The application of bioresorbable materials presenting shape memory temperature close to body temperature additionally should simplify construction and increases reliability of stent delivery systems.

The main goal of our research is to obtain such a system for implantation of bioresorbable vascular stents, which will be obtained by the micro-injection moulding, additionally self-expanded because their shape memory behaviour induced by increase of temperature generated on the guide catheter. In this paper the results of optimization studies of the composition of the polymer, which will be used for forming the stents as well as results of designing of their target shape are presented.

Materials and Methods

L-Lactide, glycolide (Glaco Ltd. China) and trimethylene carbonate -TMC (Foryou Medical Devices Co. Ltd. China) were purified by recrystallization from ethyl acetate solution and dried in a vacuum oven at room temperature. Initiators and catalysts: zirconium(IV) acetylacetonate, zinc(II) acetylacetonate monohydrate, 1,4-butanediol (Aldrich Corp.) were used as obtained. The "primitive" stents was obtained with using devices set; Thermo Scientific HAAKE MiniLab & HAAKE MiniJet.

Results and Discussion

On the basis of previous studies [3,4], the terpolymer of L-lactide, glycolide and trimethylene carbonate, obtained via Ring Opening Polymerization (ROP) of L-lactide and glycolide, conducted with presence of the aliphatic oligocarbonate as a macroinitiator was the selected material designed to forming the stents. The macroinitiator is also obtained by ROP of the cyclic TMC catalysed with zinc compound in the presence of 1,4-butanediol. The resulting oligomer having an average weight of about 6,000 g/mol does not contain more than 1-2% of monomer, and the chains are terminated with hydroxyl groups at both ends. Without further purification, this oligomer was used in the second stage of the synthesis, played role of macroinitiator. Prepared on this way final triblock terpolymer shows a specific construction of the chain, which defines the central block carbonate connected by both sides with the L-lactide/ glycolide copolymer chains presented segmented microstructure. This special terpolymer chain structure resulted good mechanical properties and shape memory behaviour induced by temperature slightly higher than body. The technology of synthesis of the terpolymer in a bench scale was elaborated, what allowed to produce a suitable amount of the polymer granulate to further processing. With using laboratory injection moulding machine were obtained the first "primitive" model of the stent in order to develop the procedure of crimping on the catheter and for monitoring the phenomenon of self-expansion of the stent caused by the temperature rise. Then, using the obtained data the optimizing of the geometry of the target stent was carried out, taking into account the stress state in the proposed element, the processing conditions and the planed method of implantation too. The design of the stent shape was completed on the basis of the analysis of strength properties, thermal analysis and coupled with the use of numerical modelling with finite element method [5]. The resulting final optimal shape of the stent was the basis to manufacture of precise injection mould with dimensions tolerance of less than 0.02 mm. The works on its manufacturing are currently being finalized. Soon we will begin the attempts of receiving the target stents, with using already specially prepared microinjection moulding machine - MicroPower 15 (Wittmann-Battenfeld GmbH). This device makes it possible to obtain mouldings of small dimensions and mass below one gram with the accuracy of a few microns.

Conclusions

At the present stage of research the primitive model of stents was formed with using the injection moulding technology. Synthesized by us biodegradable L-lactide/glycolide/trimethylene carbonate terpolymer turns to be fully capable to this type of processing and the final shape was at the same time the programmed permanent shape. Developed the ways of crimping stent on the catheter and set the rise temperature to about 41°C permitting its self-opening within a few seconds. With the use of numerical modelling, optimal shape of the stent was designed, which was used next for the implementation of the appropriate injection mould.

Acknowledgments

A financial support has been provided by the National Center for Research and Development, project no. PBS3/A9/38/2015 – BSM Stent "Obtaining of self-expanding, polymeric and biodegradable, drug-eluting vascular stents".

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RECORDING OF BASAL CALCIUM LEVELS IN CELLS ON GEOMETRIC STRUCTURES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 42]

Introduction

Topographical surface modifications are of importance for the integration of orthopedic and dental implants in bone tissue. Bone-building cells, the osteoblasts, recognize the underlying topography on an nm and µm scale [1]. However, it has remained unclear how the cells recognize this and why the surface topography influences cell physiology. Stochastically raw metal surfaces, such as those which are produced by corundum blasting, can both change cellular adhesion and alter the receptor expression of the integrins, the important „anchor" for binding to the extracellular matrix [2]. In order to reduce the complexity of these material surfaces and to be able to better elaborate the cell-physiological phenomena *in vitro,* we used geometrical micro-pillars. Interestingly, we were able to ascertain that osteoblasts attempt to internalize the micro-pillars, which are characterized by sharp edges and ridges [3]. This process causes not only higher energy metabolism (loss of adenosine triphosphate (ATP)) in the osteoblasts, but also the production of reactive oxygen species (ROS) in the cells [3]. Components present in the cell membrane, which otherwise play a role in the cellular intake of micro- and nanoparticles, are involved in these processes, including caveolin-1 as an important membrane-bound protein in the caveolae, cholesterol and CD68 [3]. Intracellular signals are significantly reduced and delayed in cells on micro pillars; this becomes evident by means of the intracellular mobilization of calcium (Ca^{2+}) [4]. The cells attempt at phagocytosis of the micro-pillars is, futile because the pillars are fixed in place. As a consequence of this entire process, the bone-specific cell functions are significantly inhibited, i.e. type I collagen, fibronectin and further proteins designated for the building up of the bone matrix are produced in reduced quantities [3,4]. It raises the question whether the dynamic of intracellular calcium ions (Ca^{2+}) as "second messengers" are important for the cell-material interaction.

Materials and Methods

Silicon wafers with final 100nm titanium coating (Si-Ti, 10 x 10 mm²) with a geometric array of micro pillars 5 x 5 x 5 µm (P-5x5, FIG. 1) were compared to planar surfaces (Ref). The micro pillars were fabricated by Deep reactive Ion Etching (DRIE) [3,4]. Osteoblasts (MG-63, ATCC® CRL-1427™) were cultured in DMEM with 10% FCS (PAA). The calcium imaging were performed with adherent osteoblasts (24 h growth) [4] as well as with cells attached for 10 min. (FIG. 2). For the 24h-adhesion approach the cells were cultured on wafers for 24h and afterwards stained with the calcium indicator Fluo3/AM (5 µM, 40 min incubation). For the 10min-adhesion approach; suspended cells were loaded with Fluo3/AM (5 µM) for 40 min. Afterwards the stained cells were seeded on wafers and cultured for 10 min. The recording of the cell's basal calcium level was done by confocal microscopy (LSM780, Carl Zeiss, Zen2011 (black edition) software) using a time series of 90 cycles each 2 s. For statistical analyses SPSS (15.0) was utilized with Kolmogorov-Smirnov test followed by Kruskal-Wallis test.

Results and Discussion

In 24h-adherent osteoblasts on micro-pillars an altered basal calcium signal could be observed; the cells showed decreased intracellular calcium dynamics and concentration (FIG. 3A) compared to Ref. In contrast, 10min-adherent cells exhibited a weak but stable basal calcium level on pillars comparable to the Ref (FIG. 3B). Our current approaches indicated a low basal calcium level which was independent of the topography within the first minutes of adhesion. It is possible that the proof of the low calcium level occurred on account of the missing contact via gap junctions to other cells [5]. After 24 hours, the influence of the micro-pillars due to weak calcium signal was clearly detectable. In previous studies we showed altered actin organization in short fibers on the top of micro-pillars [3,4]. It is known that an intact actin cytoskeleton affects the calcium dynamic [6].

Conclusions

Adherent osteoblast on micro-pillars with impaired cell showed a reduced basal calcium level. Investigations of the influence of topography on intracellular calcium signaling provide new insights into how external signals from physico-chemical environment affect cell behavior and finally the cell function. The understanding of the biocomplexity of cellular pathways is a challenge and of clinical relevance for the development of bio-functional implant surfaces.

Acknowledgments

SSt and CM were gratefully supported by the DGF GRK 1505/2 *welisa* as well as by DFG NE 560/7-2.

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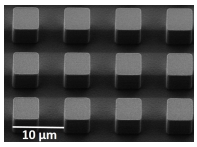

FIG. 1. SEM image of micro-pillars (P-5x5). (FE-SEM Supra 25, Zeiss, 3 kV, magnification = 2,000x, bar 10 µm).

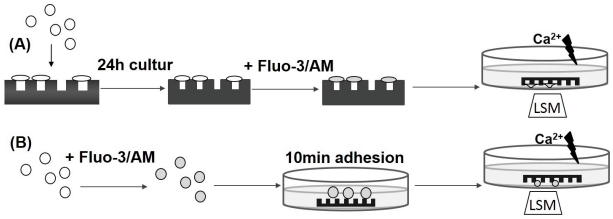

FIG. 2. Schematic illustration of calcium imaging in (A) 24h-adherent and (B) 10min-adherent cells.

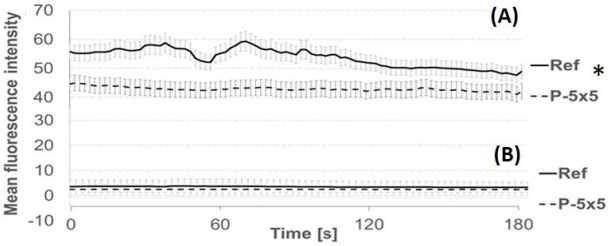

FIG. 3. Basal calcium level in (A) 24h-adherent and (B) 10min-adherent cells on micro-pillars (P-5x5) as well as planar surfaces (Ref). (LSM 780, calcium signal intensity, mean ± SD, n = 140, *p < 0.05, Kruskal-Wallis test).

CHARACTERIZATION OF CHITOSAN, COLLAGEN, HYALURONIC ACID BLENDS CROSSLINKED BY TANNIC ACID ADDITION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 43]

Introduction
Collagen, chitosan and hyaluronic acid are biopolymers widely used in biomaterials science [1]. Nevertheless materials obtained from them are not stable in aqueous environment and have to be cross-linked [2]. Moreover the modification of materials leads to the improvement of mechanical properties [3]. Tannic acid is a natural compound which can be used as cross-linking agent for the biomaterials properties modification [4].

Materials and Methods
Collagen (Coll) was isolated from rat tail tendons. Chitosan (CTS) and hyaluronic acid (HA) were purchased (Sigma-Aldrich, Poland). Collagen and chitosan were prepared as 1% solutions in acetic acid. Hyaluronic acid was dissolved in hydrochloric acid in 1% concentration. Coll and CTS were mixed in the weight ratio 50/50 and then 1, 2 and 5 wt% of hyaluronic acid was added. To the mixture 2, 5, 10 and 20 wt% of tannic acid was added as cross-linking agent. Mixtures were frozen in -80^{o}C and lyophilized. Porous structures called scaffolds were obtained and characterized by mechanical testing and infrared spectroscopy. Moreover porosity and density were measured.

Results and Discussion
Results show that after the addition of tannic acid the properties of material are modified. Young modulus increases after the addition of tannic acid. The infrared spectroscopy analysis shows that after the addition of cross-linking agent characteristic peaks from Amide A, I, II and III are shifted. It suggests that new interactions between polymers and tannic acid are present. Porosity decreases and density increases after the addition of tannic acid because the structure of composites was changed as a result of cross-linking process.

Conclusions
Tannic acid can be used as cross-linking agent where its addition modifies the properties of biopolymer material. It enhances the mechanical parameters, modifies porosity and density.

Acknowledgments
Financial support from the National Science Centre (NCN, Poland) Grant No UMO-2013/11/B/ST8/04444 is gratefully acknowledged.

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THE VITAMIN B_1 RELEASE FROM COLLAGEN, CHITOSAN AND HYALURONIC ACID BLENDS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 44]

Introduction

Natural polymers are used for preparation of several materials for biomedical application. Collagen, chitosan and hyaluronic acid are biocompatible, biodegradable and non-toxic for human body [1]. The new aspect of biomaterials science is the use of polymeric materials as matrixes for the active compounds release [2]. Such systems enhance the efficiency of medical treatment because active compounds are incorporated directly in the target site of action [3].

Materials and Methods

Collagen (Coll) was isolated from rat tail tendons. Chitosan (CTS) and hyaluronic acid (HA) were purchased (Sigma-Aldrich, Poland). Collagen and chitosan were prepared as 1% solutions in acetic acid. Hyaluronic acid was dissolved in hydrochloric acid in 1% concentration. Coll and CTS were mixed in the weight ratio 50/50 and then 1, 2 and 5 wt% of hyaluronic acid was added. To the 15 ml of polymeric mixture vitamin B_1 was added. Mixtures were frozen in -80^{o}C and lyophilized. Scaffolds were immersed in PBS solution (pH=7.4). After 2, 3, 4, 24 and 48 h, the volume 3 ml of solution was taken and replaced by the fresh PBS. The concentration of released vitamin was analysed by spectrophotometer with the use of standard curve method.

Results and Discussion

Results show that the concentration of released vitamin B_1 increases with increasing time of immersion. After 48 h the decrease of released rate was noticed. It is proper for the use of polymeric matrixes as drug delivery systems because drug should be released mostly in the initial time and then the released rate should decrease. The concentration of vitamin B_1 depends on the polymeric matrix content.

Conclusions

Polymeric matrixes based on chitosan, collagen and hyaluronic acid blends can be potentially applied as drug delivery systems what can significantly modify the therapy efficiency.

Acknowledgments

Financial support from the National Science Centre (NCN, Poland) Grant No UMO-2013/11/B/ST8/04444 is gratefully acknowledged.

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POLYESTER-CAMPTOTHECIN CONJUGATES FOR CONTROLLED RELEASE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 45]

Introduction

Camptothecin (CMPT) (FIG. 1) belongs to the family of monoterpene indole alkaloids, isolated from *Camptotheca acuminata* tree [1]. CMPT and its derivatives commonly used in antitumor therapy, exhibit a broad range of antitumor and antileukaemia activity leading to the inhibition of topoisomerase I, subsequent damage of DNA and thus cell death. However, the clinical use of CMPT is limited by its low solubility in water, high toxicity and inactivation through lactone ring hydrolysis at a physiological pH [2]. From this point of view, the disadvantage of CAMPT might be overcome by its attaching to the macromolecular conjugates [3-4].

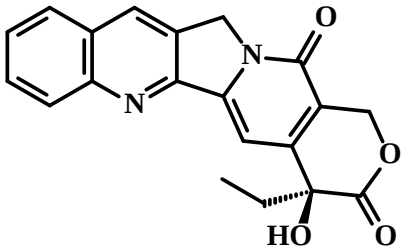

FIG. 1. Camptothecin.

The aim of this study was to prepare new polyester conjugates of CMPT. The polyester matrices were obtained by the ring-opening polymerization (ROP) of ε-caprolactone (CL), glycolide (GL) and *rac*-lactide (LA) in the presence of a new zinc-catalytic system. The preliminary studies of the influence of the polymeric chain structure on the release process of CMPT were described.

Materials and Methods

The ROP of cyclic esters was carried out under an argon atmosphere. The toxicity test was carried out according to the procedure described in our early paper [5]. Polymeric conjugates of CMPT were synthesized using 1,6-diisocyanatohexane (HDI) as linker under argon atmosphere. The polymerization products were characterized by means of ^{1}H- and ^{13}C-NMR (300 MHz, recorded in $CDCl_3$) spectroscopy. Number-average molecular weight (M_n) and polydispersity were determined by gel permeation chromatography (GPC). The zinc concentration in the obtained polymers was determined by Flame Atomic Absorption Spectrometry. The *in vitro* release study of CMPT from the synthesized conjugates was investigated by measuring the concentration of CMPT released at pH 7. The absorption in a buffer solution was determined by a UV-Vis spectrophotometer at the absorbance peak with a wavelength at 355 (lactone form) or 368 nm (carboxyl form) [4].

Results and Discussion

The macromolecular conjugates of anticancer drug were obtained from the copolymers of CL, LA and GL. The polymeric matrices and CMPT were coupled *via* HDI. The ROP process was carried out in the presence of diethylzinc/phenylalanine catalytic system (FIG. 2).

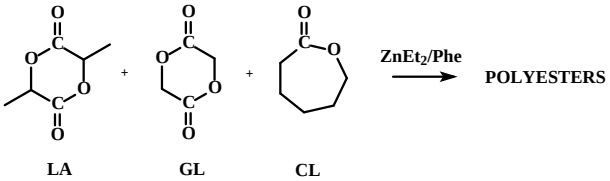

FIG. 2. Scheme of polyester matrices synthesis.

The M_n values of CL/LA and GL/LA copolymers determined by the GPC were in the range of 1900-6800 g/mol and 1600-5700 g/mol, respectively.

The cytotoxicity evaluation of the obtained polymers was studied using the luminescent bacteria *V. fischeri*. It was found that the obtained matrices were not toxic to the test biont. Four kinds of macromolecular conjugates of CMPT were prepared from CL/LA (63:37), CL/LA (42:58), GL/LA (56:44) and GL/LA (32:68). *In vitro* CMPT release from the macromolecular conjugates was carried out in PBS buffer at 37°C for 4 weeks. The percentage of the CMPT released after 4 weeks of incubation was about 62% for GL/LA (56:44), 57% for GL/LA (32:68), 42% for CL/LA (42:58) and 33% for CL/LA (63:37). The rate of *in vitro* drug release increased as the GL or LA content in the chain of copolymers decreased.

Conclusions

The synthesized biodegradable polymeric matrices were not toxic. The rates of CMPT release were shown to be directly dependent on the nature of the obtained copolymers. The kinetic rates of CMPT released were seen to be faster for the polymeric conjugates contained GL units as compared to those with CL units. Importantly, in some cases, drug "burst release" was not observed during the degradation process. The obtained results demonstrate that the macromolecular conjugates are interesting and promising materials for the controlled release of CMPT.

Acknowledgments

This work was financially supported by National Science Centre of Poland (Project NCN OPUS-5 DEC-2013/09/B/ST5/03480 entitled: "Elaboration of anti-cancer drug implantational delivery system immobilized on polymer matrix").

This work was also financially supported by the Medical University of Warsaw (The Student Grant – Mini-grant Studencki WUM 2015, Urszula Luchowska: "Innowacyjne biodegradowalne nośniki cytostatyków - synteza i badania strukturalne").

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THE PROPERTIES OF COLLAGEN/CHITOSAN POROUS MATRICES IN THE PRESENCE OF SMALL AMOUNT OF POLY(ETHYLENE)GLYCOL

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[ENGINEERING OF BIOMATERIALS 138 (2016) 46]

Introduction

Collagen is an especially abundant protein in animals. It is the main protein of connective tissue. Collagen is readily available and it possesses many interesting properties, such as biocompatibility, non-antigenecity, non-toxicity. For this reason, this protein is regarded as one of the most important and useful biopolymer in biomaterial's research [1]. Collagen based materials are widely used in tissue engineering. However, the disadvantage of using collagen as a biomaterial for tissue repair is its high degradation rate, which leads rapidly to a loss of mechanical properties [2] Many attempts have been made to overcome this problem through the means of mixing collagen with either natural (e. g. chitosan) or synthetic polymers or different crosslinking method [3]. Chitosan is a natural polymer (polysaccharide) prepared from chitin by deacetylation. Chitosan-based materials possess high biocompatibility and various biological functions such as wound healing, antibacterial activity [4]. The aim of present study was to investigate the influence of addition a small amount of poly(ethylene)glycol on the properties of collagen/ chitosan porous matrices.

Materials and Methods

Collagen (col) was obtained in our laboratory from the tail tendons of young rats. Chitosan (chit) and poly(ethylene)glycol (PEG) were supplied by the company Sigma–Aldrich. In this work a porous collagen/chitosan/poly(ethylene) glycol matrices were fabricated by the freeze-drying method. Firstly, collagen solution with concentration of 1% was prepared from lyophilized collagen in deionized water using an IKA disintegrator. Then, 1% chitosan solution in acetic acid and 2% PEG solution were prepared. Polymeric blends were obtained by mixing suitable volumes of chitosan, collagen and PEG solutions and the final weight ratio were presented in TABLE 1.

TABLE 1. The composition of studied samples

	Sample
1	col
2	chit
3	col50/chit50
4	col25/chit75
5	col75/chit25
6	col50/chit50 + 5% PEG
7	col75/chit25 + 5% PEG
8	col25/chit75 + 5% PEG

In order to improve especially the mechanical properties and susceptibility to degradation of the materials, the samples were physically modified using a dehydrothermal treatment (DHT). For DHT crosslinking, freeze–dried samples were placed under a vacuum at a temperature of 110°C for 24 h.
The effects of poly(ethylene)glycol addition was examined using measurements: water uptake ability, porosity and mechanical properties.

Results and Discussion

FIG. 1 shows SEM images of the horizontal cross section of freeze-dried samples.

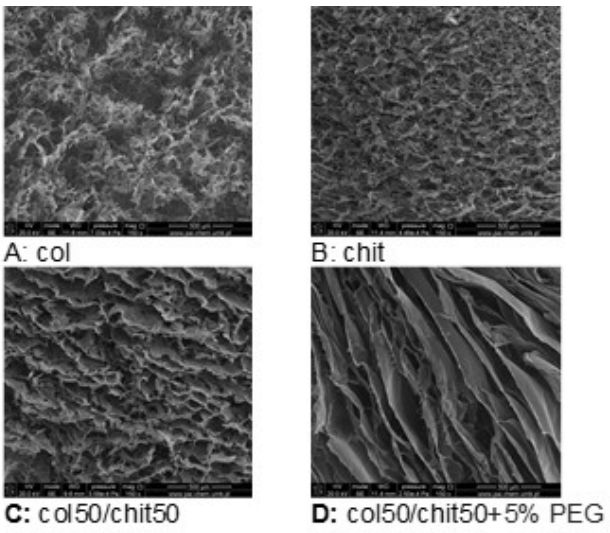

FIG. 1. SEM images of different porous matrices.

The samples prepared by freeze-drying resulted in porosity from 63.5% (chit) to 83.8% (col25/chit75) (TABLE 2). Compressive moduli (E_c) of prepared samples are shown in TABLE 2. The addition both chitosan and PEG gives rise to an increase in the stiffness of samples which enhanced the values of the mechanical characteristics.

TABLE 2. Porosity (ϵ) and compressive modulus [E_c] of different col/chit and col/chit/PEG matrices.

Sample	E_c [kPa]	ϵ [%]
col	3.61	78.5
chit	21.8	63.5
col75/chit25	14.4	80.2
col50/chit50	10.4	81.3
col25/chit75	17.5	83.8
col75/chit25 + 5% PEG	14.8	75.5
col50/chit50 + 5% PEG	9.18	66.7
col25/chit75 + 5% PEG	17.7	78.3

The samples containing collagen and chitosan show a great ability to absorb water (results not shown) and have the highest degree of porosity and good mechanical properties.

Conclusions

The addition of PEG caused the reduction of the degree of porosity and the degree of swelling but it led to the increase of the value of the degree of enzymatic degradation.

Acknowledgments

Financial support from the National Science Centre (NCN, Poland) Grant No UMO-2013/11/B/ST8/04444 is gratefully acknowledged.

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SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF THE BIODEGRADABLE POLYESTER/BISPHOSPHONATE CONJUGATES FOR COATING OF THE APATITE MATERIALS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 47]

Introduction

Biomaterials are widely used in medicine and pharmacy nowadays [1]. The development of composites is one of many possibilities widespread use of biomaterials. Especially promising are biodegradable polymer/ hydroxyapatite composites that can be used for filling bone or tooth defects as well as drug carriers in a targeted therapy [2]. Disodium pamidronate (PAM) belongs to the second generation of bisphosphonates. PAM disturbs the function of osteoclasts, thereby inhibiting bone resorption of osteoclast. It is used to treat Paget's disease and osteoporosis [3-5]. Furthermore, the presence of an amino group in the PAM molecule allow to obtain the biodegradable polymeric carrier/PAM conjugate as a component of the implantation therapeutic system. Therefore, the aim of this study was the synthesis and physicochemical and biological characterization of the copolymeric carriers obtained through the ROP of a cyclic ester in the presence of 2-hydroxyethyl methacrylate (HEMA) and hyperbranched bis-MPA initiators; covalent conjugation of the PAM to the synthesized matrices as well as the coating of the porous hydroxyapatite doped with selenium ions by the synthesized conjugates.

Materials and Methods

The ROP of cyclic esters was carried out under an argon atmosphere. The cytotoxicity test was carried out according to the procedure described in our previous paper [6]. The copolymeric conjugates of PAM were synthesized by multi-step chemical synthesis. The copolymerization products and conjugates were characterized by means of ^{1}H- and ^{13}C-NMR (300 MHz, recorded in DMSO-d_6 spectroscopy). Number-average molecular weight (M_n) and polydispersity were determined by gel permeation chromatography (GPC).

Results and Discussion

The biodegradable copolymeric matrices were prepared though the ROP of ε-caprolactone (CL) and L,L-lactide (LLA) in the presence of linear and branched initiators. The cytotoxicity of the synthesized copolymers was evaluated with a bacterial luminescence test and protozoan assay, which showed that the obtained polymers were not cytotoxic. The M_n values of CL/LA copolymers determined by the GPC were in the range of 11800-21000 g/mol. The macromolecular conjugates of bisphosphonate were obtained from the synthesized copolymers and PAM. The drug was coupled to the copolymeric carrier by an amide bond (FIG. 1).

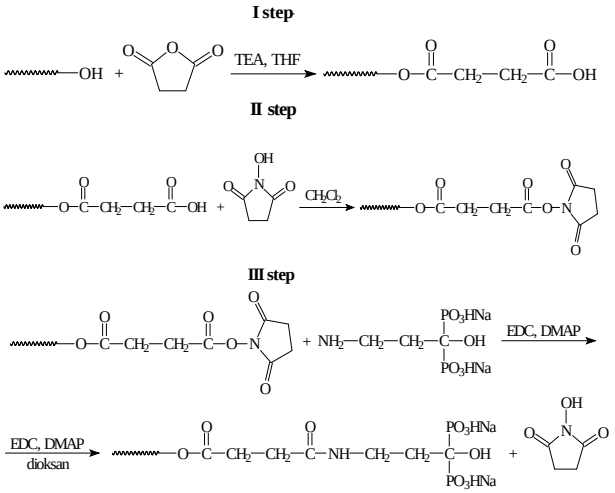

FIG. 1. The scheme of the synthesis of the biodegradable copolymer/PAM conjugates.

In the first step of this synthesis, the addition of succinic anhydride to the copolymer chain in the presence of TEA catalyst was carried out. The next step has led to the functionalization of the terminal carboxyl group of the copolymer chain with *N*-hydroxysuccinimide. The third and final step consisted covalent conjugation of the PAM to a functionalized copolymer carrier. The reaction was carried out in the presence of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide and 4-dimetyoaminopirydyna catalyst. The synthesized macromolecular conjugates were characterized by ^{1}H- and ^{13}C-NMR spectroscopy.

Conclusions

As a result of this research, the biodegradable copolymeric matrices were synthesized though the ROP of cyclic esters and spectrally characterized. The biodegradable copolymeric conjugates of PAM were obtained in the further step of this work. A new amide bond was formed between the hydroxyl end-groups of the synthesized copolymer matrices and an amine group of bishosphonate. The structure of the polymeric conjugates was characterized by various spectroscopy techniques.
The porous hydroxyapatite doped with selenium ions was coated with the synthesized conjugate in the last step of this work. The *in vitro* release profile of PAM is currently carried out in our laboratory.

Acknowledgments

This work was financially supported by National Science Centre of Poland (Project NCN DEC-2011/03/D/ ST5/05793; "Synthesis and characterization of polymer-apatite composite containing selenium and bisphosphonates").
This work was also financially supported by the Medical University of Warsaw (Mini-student grant, Katarzyna Orłowska, FW23/NM1/2015, "Synthesis of new conjugate – biodegradable poliester/bisphosphonate as a part of the polymer-apatite composite containing selenium").

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EFFECT OF FIBRIN-NANOCOATING OF NANOFIBROUS POLYMER MEMBRANES ON THE ADHESION AND PROLIFERATION OF HUMAN DERMAL FIBROBLASTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 48]

Introduction

Our research aims to construct appropriate scaffolds for healing the skin damage. Nanofibrous membranes seem to be promising for fabrication of advanced bilayered skin substitutes. The structure of biodegradable synthetic nanofibrous membranes well simulate natural composition of extracellular matrix and by that enhance communication between cells. Moreover, adhesion-mediating molecules can be adsorbed in a right conformation on nanostructured membranes and therefore can be better recognized by the cells integrins [1]. Our study contributes to the basic research in the field of molecular mechanisms of adhesion, proliferation and phenotypic maturation of dermal fibroblasts and the control of their behavior through the extracellular matrix, represented by fibrin.

Materials and Methods

Nanofibrous polylactid acid (PLA) membranes were prepared by needle-less electrospinning technology. The membranes were further coated with fibrin by using different preparation methods and subsequently fibronectin was attached in order to enhance cells affinity to the colonizing material. Adhesion and growth of human dermal fibroblasts, i.e. major cell type of dermis, were evaluated on these nanocoated membranes by MTS assay and by immunofluorescence staining. Relative expression of collagen I mRNA was measured by real-time PCR and also collagen I protein production was observe by immunofluorescence staining. Structure of fibrin-nanocoating was characterized by scanning electron microscopy (SEM). The quantitative data is presented as mean ± standard deviation (S.D.) or standard error of mean (S.E.M) from three independent samples for each experimental group and time interval. Statistical significance was evaluated using the Kruskal-Wallis test. Values of $p \leq 0.05$ were considered as significant.

Results and Discussion

The results show that the fibrin-modified membranes improved adhesion and proliferation of human dermal fibroblasts. Furthermore, the morphology of fibrin modification seems to be crucial for the fibroblasts adhesion and consequently for their phenotypic maturation. Fibrin either formed coating around individual fibers in the membrane (FIG. 1A), or created thin nanofibrous mesh on the whole surface of the membrane (FIG. 1B), which depended of the method of fibrin preparation. Fibroblasts on the membranes with fibrin distributed around the fibers remained in their typical spindle-like morphology while the cell behaviour on thin fibrin mesh on the membrane was absolutely different. The cells were more spread in all directions and moreover their proliferation was slightly higher. Fibronectin created secondary mesh on primary fibrin mash and enhanced the cell attachment and also the cell growth. Relative expression of collagen I mRNA and also protein production were higher on the fibrin mash in compared with fibrin distributed around the fibers.

Fibrin generally improved fibroblasts adhesion and proliferation which correlate with previous works [2], but the fibrin homogenous mesh probably provides better conditions for the cell attachment. This accelerates fibroblasts growth and production molecules of ECM.

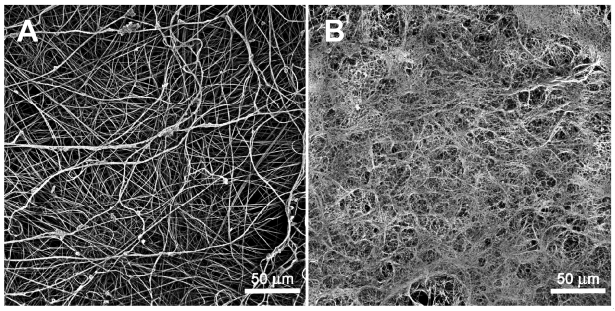

FIG. 1. Morphology of fibrin-nanocoating. Fibrin around the fibers (A) and fibrin mesh on the membrane surface (B). Fibrin was stained by immunofluorescence using primary and secondary antibody conjugated with Alexa Fluor 488. Leica TCS SPE DM2500 confocal microscope, obj. 40x/1.15 NA oil.

Conclusions

The PLA membranes modified with fibrin homogenous mesh are promising for construction of advanced bilayered skin substitutes. They enhanced the adhesion, proliferation and ECM production by dermal fibroblasts.

Acknowledgments

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THE MECHANICAL PROPERTIES OF GELATIN/ALGINATE HYDROGELS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 49]

Introduction

Gelatine is a product of thermal denaturation or partial hydrolysis of insoluble collagen, which forms thermoreversible physical gels in hydrogen-bond friendly solvents. It possesses amino acid composition typical for collagen (Gly-X-Y) and is known as non-immunogenic material. However, in contrast to collagen, it is susceptible to most proteases [1].

The alginate is the anionic polysaccharide derived from brown algae. It creates irreversible gels in the presence of divalent cations such as Ca^{2+} [2].

Biopolymeric hydrogels, due to their biocompatibility, biodegradability and non-toxicity for human organism, are attractive biomaterials for medical and tissue engineering applications.However, often they suffer from the lack of mechanical strength, hence limiting their use [3].

The aim of the study was to obtain and characterize the mechanical properties of gelatine/alginate hydrogels cross-linked by calcium ions.

Materials and Methods

20% gelatine solution and 8% alginate solution were mixed at various weight ratios of dry polymers (95/5, 90/10, 80/20, 70/30, 50/50). Then the blends were poured into molds and cross-linked by 8% calcium chloride solution.

The gels were mechanically tested (tensile and compressive strength) using Zwick&Roell Z0.5 machine.

Results and Discussion

The mechanical properties of gelatine/alginate gels depend on the mixture composition. The gels with the higher alginate content are more resistant to compression (FIG. 1).

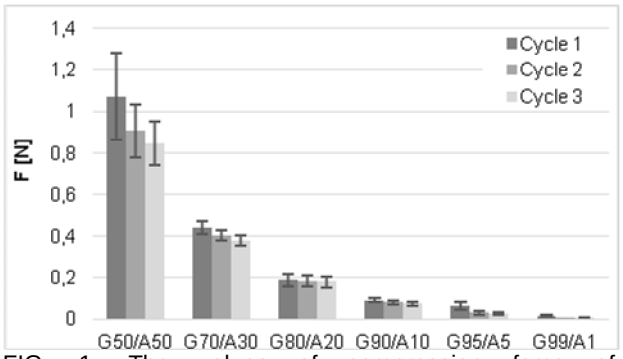

FIG. 1. The values of compression force of gelatin/alginate gels cross-linked with calcium chloride.

Also the tensile strength and Young's modulus increase for the gelatin/alginate gels with the growing amount of polysaccharide (FIG. 2 and 3).

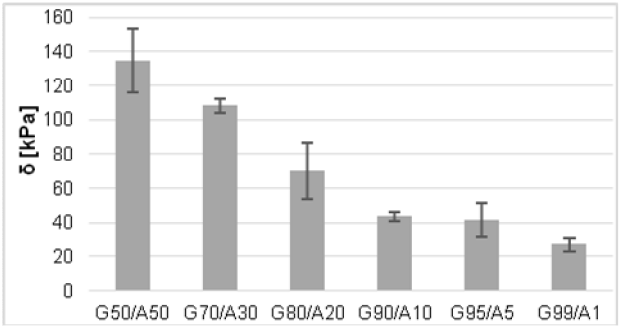

FIG. 2. The values of tensile strength of gelatin/alginate gels cross-linked with calcium chloride.

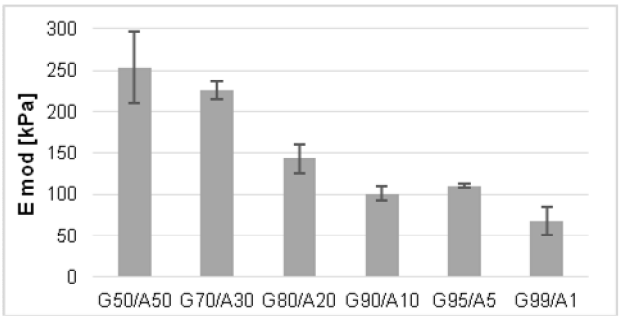

FIG. 3. The values of Young's modulus of gelatin/alginate gels cross-linked with calcium chloride.

There are ionic interactions between anionic groups of alginate and cationic amino acids residues of gelatine. However, the cross-linking using calcium chloride plays a crucial role to mechanical properties improvement of gelatine/alginate hydrogels.

Conclusions

The compressive and tensile strength of the gelatine/alginate gels cross-linked by calcium ions increase with the higher content of polysaccharide in the mixture.

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BIOELECTROCHEMICAL PROPERTIES OF Ti13Nb13Zr ALLOY BEFORE AND AFTER SURFACE MODIFICATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 50]

Introduction

The osseointegration process of biomedical titanium alloys is affected by factors such as surface of the implant, its biocompatibility, method of modification, bone bed, shape of the implant and load conditions. Smooth surfaces of titanium and its alloys have been reported to be less desirable for bone fixation than after surface modification. Roughened surface of titanium implants has shown better osteogenic activities such as cell attachment, cell proliferation, adsorption of protein and calcium deposition, proving to be beneficial for osseointegration. Surface modification resulting in a change of physical properties also entails a change in the chemical properties of the surface. The implant acts on the surrounding tissue by its surface. This interaction is related to the interaction of biological fluids with the surface of the implant [1-3].

Materials and Methods

The aim of this study was to obtain three types of modified surfaces on the Ti13Nb13Zr alloy for biomedical applications. First sample was mechanically polished using abrasive papers only. Second sample was subjected to anodic oxidation in order to form TiO_2 nanotubes at room temperature in 1 M $(NH_4)_2SO_4$ with 2 wt.% of NH_4F addition at the potential of 20 V for 120 min. Third sample was after anodization with formed TiO_2 (rutile) nanotubes on the surface with additionally embedded nanoparticles of silver by immersion in 1 M $AgNO_3$ solution for 30 min and subsequent photocatalytic reduction of Ag^+ ions to metallic form using ultraviolet radiation for 30 min. Surface morphology observations of the Ti13Nb13Zr alloy before and after such surface modifications were carried out using a scanning electron microscope with field emission. Chemical composition analysis was performed by energy dispersive spectroscopy. X-ray studies of the modified Ti13Nb13Zr alloy were realized in the grazing incidence X-ray diffraction geometry. The XRD pattern of the starting material was measured in the classical Bragg-Brentano geometry. Zeta potential measurements for the tested materials were carried out in aqueous electrolytes with different ionic strength using electrokinetic analyzer. The following solutions were applied: potassium chloride (0.001 mol/l), phosphate buffered saline (0.001 mol/l and 0.01 mol/l), and artificial blood (0.01 mol/l).

Conclusions

It was found that the bioelectrochemical properties of the Ti13Nb13Zr alloy surface carried out before and after the surface modification can be determined using the zeta potential in a biological environment. It was ascertained that there is an effect of the electrolyte and the ionic strength on the zeta potential of the investigated surfaces. The obtained results suggest that the proposed method of surface modification is promising for better osseointegration of the Ti13Nb13Zr implants.

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CHITOSAN/β-1,3-GLUCAN/HA BONE SCAFFOLD POSSESSES OSTEOPROMOTIVE PROPERTIES IN VITRO

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[ENGINEERING OF BIOMATERIALS 138 (2016) 51]

Introduction

Osteoblast differentiation may be divided into 3 sequential phases: proliferation, extracellular matrix (ECM) synthesis, and ECM mineralization [1]. Each abovementioned phase is characterized by different bone alkaline phosphatase (bALP) activity, osteocalcin (OC) and type I collagen (Col I) synthesis [1]. Our previous reports [2,3] describe fabrication and basic characterization of novel tri-component bone scaffold composed of krill chitosan, bacterial β-1,3-glucan (curdlan), and hydroxyapatite (HA) for bone tissue engineering application. The aim of this work was to assess osteogenic potential of novel chitosan/β-1,3-glucan/HA scaffold via detection of differentiation stage-specific markers expressed by osteoblastic cell lines in contact with novel biocomposite. The typical markers of the first (Col I), second (bALP), and third stage (OC) of osteoblast differentiation process were evaluated during *in vitro* experiment [4].

Materials and Methods

Cell culture. The study was carried out using normal human foetal osteoblasts (hFOB 1.19) and mouse calvarial preosteoblasts (MC3T3-E1 Subclone 4).

Osteogenic differentiation assessment. Osteoblasts were seeded directly on the scaffold and cultured in osteogenic medium for 20 days. Two-dimensional osteoblast culture (cells on flat polystyrene plate) served as a control. Osteoblast differentiation process was monitored at predetermined time intervals: 4, 14, and 20 days. The bALP and OC were determined using enzyme-linked immunosorbent assays (ELISA) and Col I was evaluated qualitatively by direct immunofluorescence (DIF) method using confocal microscope. The experiments were conducted in quadruplicate (4 different samples were tested).

Statistical analysis. The unpaired t-test was performed to assess statistical differences between the results obtained with the control cells and the cells cultured on the tested scaffold.

Results and Discussion

The bALP ELISA test demonstrated that novel scaffold had ability to significantly increase bALP activity in hFOB 1.19 cells (FIG. 1) [4]. The OC ELISA test revealed that MC3T3-E1 cells cultured on the novel material produced significantly more OC compared to the 2D control culture on the 20th day (FIG. 2). It clearly indicates that novel scaffold enhances differentiation of MC3T3-E1 cells via promotion of osteocalcin expression [4]. Confocal microscopy observation demonstrated that both osteoblastic cell lines cultured on the novel scaffold synthesized large amounts of Col I protein that was deposited in ECM and was successfully visualized by DIF method [4]. Unlike control cells, hFOB 1.19 cells grown on the scaffold revealed capability to form mineralized nodules what suggests that on the 20th day of the experiment hFOB osteoblasts were in more advanced stage of differentiation compared to the control cells. MC3T3-E1 cells did not form mineralized nodules but deposited meaningfully greater amounts of Col I in ECM compared to the control cells.

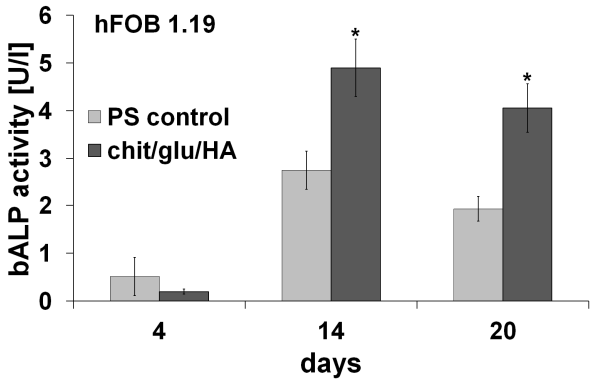

FIG. 1. The bALP activity assessed by ELISA test during osteogenic differentiation of hFOB 1.19 cells.

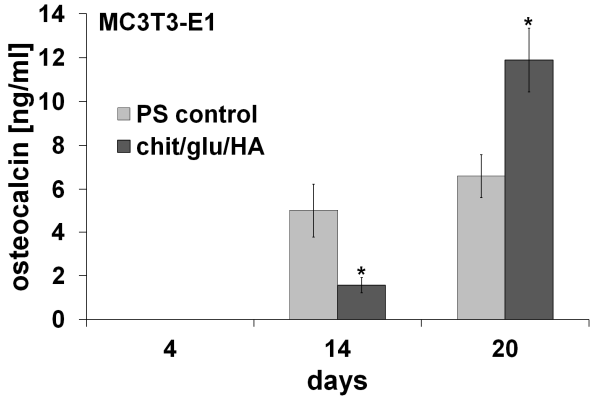

FIG. 2. The osteocalcin synthesis assessed by ELISA test during osteogenic differentiation of MC3T3-E1 cells.

Conclusions

Obtained data clearly prove that novel scaffold has ability to increase bALP activity, to enhance extracellular matrix synthesis (Col I and OC), and to induce mineralized nodule formation during osteogenic differentiation. Novel scaffold enhances osteoblast differentiation and thus possesses osteopromotive properties. Since osteopromotive properties of the material are essential for rapid new bone formation, it may be inferred that novel composite is promising biomaterial for bone tissue engineering applications to accelerate bone regeneration process.

Acknowledgments

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BIOLOGICAL EVALUATION OF β-1,3-GLUCAN/HA BONE SCAFFOLD FABRICATED VIA NEW METHOD

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[ENGINEERING OF BIOMATERIALS 138 (2016) 52]

Introduction

β-1,3-glucan (curdlan) is a bacterial linear polysaccharide composed of D-glucose units. It is found that curdlan and its derivatives have various biological activities, such as immunomodulatory, anti-cancer or anti-coagulant properties. Curdlan also possesses unique gelation ability [1,2]. To date, study carried out by our research team demonstrated that thermal method for curdlan gelation is appropriate to fabricate biocompatible materials dedicated for bone tissue engineering [3,4]. Nevertheless, fabrication process of these materials needs high temperature what limits their modification by thermo-sensitive compounds like proteins. The aim of this study was to fabricate the β-1,3-glucan/hydroxyapatite scaffold without high temperature and to assess basic biological properties *in vitro*.

Materials and Methods

1. β-1,3-glucan/hydroxyapatite (glu/HA) scaffold fabrication

Firstly, HA granules were produced in accordance with common procedure described elsewhere [5]. Then the glu/HA scaffold was produced via gelation at room temperature according to procedure described in our Patent pending [6]. After fabrication process, the glu/HA scaffold, containing 8 wt.% of curdlan and 80 wt% of HA granules was cut into suitable sizes and left to air dry.

2. *In vitro* cell culture experiments

The experiments were performed using normal mouse calvarial preosteoblast cells (MC3T3-E1 subclone 4) obtained from ATCC. The cells were cultured according to ATCC recommendation (37^{o}C; MEM Alpha supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin). For cytotoxicity determination, the MC3T3-E1 cells were seeded directly on the glu/HA scaffold at concentration of 1x10^{5} cells/disc. After 24-hour incubation the cytotoxic effect of glu/HA was evaluated quantitatively by colorimetric LDH assay and qualitatively by staining the cells with Live/Dead Double Kit. Additionally, the growth of osteoblast cells on the glu/HA scaffold was evaluated. The MC3T3-E1 cells were seeded at concentration of 5x10^{4} cells/disc. Five and eight days after cell seeding, proliferation was assessed using colorimetric WST-8 test. Moreover osteoblast cytoskeleton and nuclei was stained with AlexaFluor635phalloidin and Hoechst33342, respectively.

Results and Discussion

LDH test showed the lack of cytotoxicity of glu/HA scaffold (TABLE 1). However, after 24-hour culture, slight, but statistically significant, reduction (to approx. 88%) in cell viability was observed. Nevertheless, confocal microscopic observation (data not shown) demonstrated that MC3T3-E1 cells cultured on the glu/HA scaffold were viable, well spread and possessed typical shape, what indicates that surface of the glu/HA scaffold promotes osteoblast adhesion. Moreover, cell proliferation on the glu/HA material was assessed. After 5 days of culture, WST-8 test showed that number of cells on the glu/HA scaffold was slightly, but statistically significantly, decreased in comparison to control cells (cells grown on polystyrene). In turn, after 8-day incubation there were more osteoblasts on glu/HA scaffold compared to the control. Nevertheless, the results were not statistically significant. On the other hand, confocal microscope observation (data not shown) revealed groups of well spread cells on the glu/HA surface after 5-day culture. Cells had extensive system of cytoskeletal filaments, many filopodia, and large nuclei what proved their good proliferation on the scaffold. On the 8th day of the experiment the considerable increased in cell number was observed and almost whole surface of the glu/HA scaffold was covered by multilayer of osteoblasts.

Conclusions

Presented results clearly indicate that new method for the β-1,3-glucan/hydroxyapatite scaffold fabrication allows to obtain non-toxic and supportive to proliferation material. Moreover, new method for the glu/HA scaffold production enables its modification with thermo-sensitive biological active molecules in order to intensify bone regeneration process *in vivo*.

Acknowledgments

The study was supported by Ministry of Science and Higher Education in Poland within MNmb1 project of Medical University of Lublin, Poland. The paper was developed using the equipment purchased within agreement No. POPW.01.03.00-06-010/09-00 Operational Program Development of Eastern Poland 2007-2013, Priority Axis I, Modern Economy, Operations 1.3. Innovations Promotion.

TABLE 1. Quantitative cytotoxicity evaluation of glu/HA scaffold against MC3T3-E1 cells assessed 24 hours after cell seeding. The results were expressed as mean values ± SD. *Statistically significance obtained at $p < 0.05$ compared to the control.

Sample	Cell viability [% of control]
control (polystyrene)	100.00 ± 3.34
glu/HA scaffold	87.72 ± 6.31*

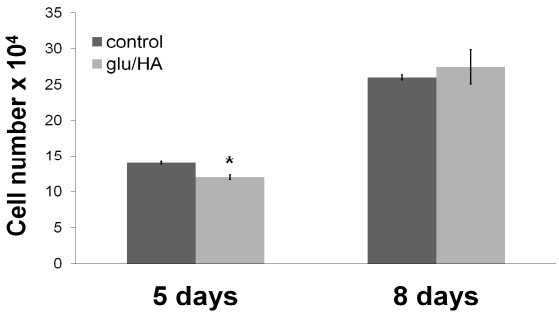

FIG. 1. Proliferation of MC3T3-E1 cells on polystyrene (control) and glu/HA scaffold after 5- and 8-day incubation. The results were expressed as mean values ± SD. *Statistically significance obtained at $p < 0.05$ compared to the control.

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SINGLE AND MULTIDRUG FILOMICELLES AS ANTICANCER DRUG DELIVERY SYSTEM

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[ENGINEERING OF BIOMATERIALS 138 (2016) 53]

Introduction

Polymeric micelles, in particular those prepared from polylactide-poly(ethylene glycol) (PLA-PEG) block copolymers, have been extensively studied as drug carrier because of many advantageous properties including bioresorbability, controlled drug release, ability to avoid reticuloendothelial system (RES) uptake, tumor targeting by enhanced permeability and retention (EPR) effect, etc [1]. Flexible worm-like "filomicelles" can be up to 8 μm long and in analogy to filoviruses – possess a long circulation time up to a week in the bloodstream because their unique visco-elastic properties and hydrodynamics could reduce interactions with the blood vessel walls [2]. The aim of this study was to evaluate the potential of bioresorbable PLA-PEG filomicelles for prolonged delivery of paclitaxel. Slow release of cytostatic drugs is very advantageous due to prolonged exposure of tumor cells to cytostatic over multiple cell cycles. This study aimed also to analyze the influence of drug-drug and drug-polymer interactions on drug loading and release properties of multidrug micelles. Simultaneous administration of two or more pharmacologically active agents with different mechanisms of action is recognized as more efficient compared to conventional therapy based on a single therapeutic agent [3]. Drug combination in anticancer treatment primarily aims to overcome tumor heterogeneity and multidrug resistance (MDR), and to achieve additive or more desirable synergistic anticancer efficacy without overlapping toxicity [3,4].

Materials and Methods

Micelles were prepared by co-solvent/evaporation method. Single drug loaded micelles were obtained with paclitaxel (Ptx) and multidrug micelles with mixture of Ptx and 17-AAG or Ptx, 17-AAG and rapamycine (Rap). The morphology of micelles was observed by TEM and AFM. NMR was applied for compositional and structural analyses of micelles in a solvated state. FTIR was used to evaluate interactions between particular drugs and between drugs and copolymer. The *in vitro* release of drugs from micelles was realized by dialysis method. *In vitro* drug release and degradation study was conducted at 37^{o}C in phosphate buffered saline (PBS) at three different pH values (pH 7.4, pH 5.5 and pH 3.0). Quantitative assessment of paclitaxel was conducted by means HPLC.

Results and Discussion

The study revealed that using PLA-PEG copolymers with the same gross composition but with different PLA configurations results in formation of micelles with different morphologies. In fact, spherical micelles were obtained for poly(DL-lactide)-poly(ethylene glycol) (PDLLA-PEG), and filomicelles for poly(L-lactide)-poly(ethylene glycol) (PLLA-PEG). Therefore, polymer chain stereoregularity seems to strongly affect the micelle's morphology.

The release of paclitaxel from one drug loaded micelles is strongly dependent on the degradation of micelles. A biphasic drug release profile is observed for both PLLA-PEG and PDLLA-PEG micelles: slow release in the first phase and faster release in the second phase. Degradation is faster at acidic pH than at pH 7.4, and PLLA-PEG filomicelles degrade less rapidly than PDLLA-PEG spherical micelles, leading to various rates of drug release.

Ptx and 17-AAG present similar loading efficiencies in double loaded micelles probably due to interactions of drugs with each other and also with the copolymer. In contrast, unequal drug loading properties are observed for triple loaded micelles. Rapamycin shows very weak interactions with the copolymer, and displays the lowest loading efficiency. For the three drugs similar release profiles are observed: a strong burst followed by slower release. Nevertheless, Ptx release from micelles is significantly slower as compared to 17-AAG and Rap, probably due to interactions of NH and OH groups of Ptx with the carbonyl group of PLA.

Conclusions

Slow and sustained release of Ptx from filomicelles was revealed and correlation of this process with degradation. In fact, using long PLLA block provides slow degradation and thus leads to slow drug release from filomicelles. Faster degradation of PDLLA block leads to faster drug release from spherical micelles. The study reveals also the crucial importance of intermolecular interactions for drug loading and release properties.

Ptx-loaded micelles and multidrug micelles exhibit advantageous effect of prolonged drug release and cytotoxic activity against Caco-2 human colorectal adenocarcinoma cell line, which makes them a promising solution for drug delivery to solid tumors.

Acknowledgments

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THE INFLUENCE OF COPOLYMER COMPOSITION AND COATING METHOD ON DRUG RELEASE FROM BIODEGRADABLE SURFACE LAYER

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[ENGINEERING OF BIOMATERIALS 138 (2016) 54]

Introduction

The development of drug eluting stents (DES) has significantly decreased the restenosis rate [1]. However, many studies have shown that the side effects of DES still remain, e.g., inflammation, late thrombosis, and late restenosis. These side effects are caused by the DES's lack of capacity for adjusting the drug dose, drug effectiveness, and release behavior according to the disease condition of the treated blood vessel [2]. Therefore, for DES, it is essential to control the dose and the release behavior of the drug. The ideal DES should have a slow and controlled drug release, with release kinetics in which the antiproliferative drug can be quickly released initially in the first week, but the total release time should be maintained for at least a month after the DES implantation [3]. There are many factors that influence the drug release from DES, such as the polymer, drug, coating methods, drug storage, elution direction, coating thickness, pore size in the coating, and release conditions as well as the influence of the hemodynamics after the implantation [4]. In this study, biodegradable drug-eluting polymer coating layer is developed. This surface layer is intended to cover biodegradable stents. The influence of copolymer composition and coating method on release rate of sirolimus has been analyzed.

Materials and Methods

Drug – eluting coating layer has been obtained from poly(lactide-co-trimethylene carbonate) (PLA/TMC) and sirolimus by air brushing and dip coating method. The morphology of coatings was observed by SEM. *In vitro* drug release study was conducted at 37^{o}C in release media consisting of 0.9% sodium chloride, 0.05% Brij 35, and 0.0003% BHT dissolved in purified water. Quantification of sirolimus was performed at the wavelength of 287 nm using a high performance liquid chromatography (HPLC) system equipped with a reverse phase C-18 column maintained at 40°C. The mobile phase consisting of methanol and 0,1% formic acid (85:15 v/v) was delivered at a flow rate of 1ml/min.

Results and Discussion

The drug release rate has become one of the important criteria for the evaluation of drug-eluting stents. The selection of the drugs and the carriers as well as the drug coating preparation process can reduce or negate the potential disadvantages of DES. In the study we analyzed the influence of several factors on release of sirolimus e.g. copolymer composition, amount of drug, coating method and concentration of drug-polymer solution used for dip coating procedure. Observations of the surface obtained by dip coating method showed that PLA/TMC forms homogenously distributed, smooth layer (FIG. 1A) with even thickness.

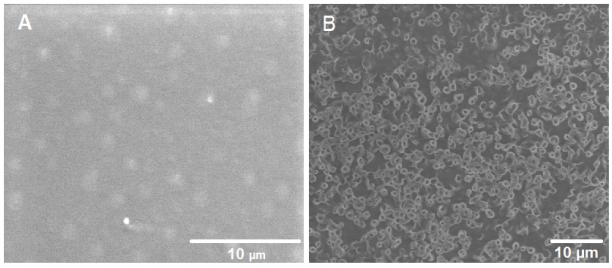

FIG. 1. SEM micrograph of PLA/TMC drug eluting layer before (A) and after 13 weeks of degradation (B).

For a biodegradable polymer, the hydrolytic cleavage of the polymer chains leads to the degradation or erosion of the matrix, which usually controls the release of drug [5]. In fact, the surface became porous during degradation (FIG. 1B), which facilitated drug release. Most of sirolimus was released until 13 weeks of degradation. The drug release and polymer erosion should be simultaneous; hence, there are not drug remnants in the tissue after hydrolytic degradation of the polymer [6].

Conclusions

The biodegradable drug eluting coating layer was developed. Regular and even release process make them a promising solution for medical applications.

Acknowledgments

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MECHANICAL HYSTERESIS TESTS FOR PORCINE TENDONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 55]

Introduction

Mechanical hysteresis of tendons describes the energy dissipated due to material viscoelastic properties. The measurement of the tendons ability to store and efficiently return energy during locomotion, allows to assess tendons thermal damage and the amount of metabolic energy that can be saved during locomotion, what is important for biomechanics of sport or rehabilitation [1-3]. The porcine tendon model is commonly used for the biomechanical investigation of various reconstruction techniques of tendon grafts [4].
The study was conducted to determine hysteresis of porcine tendons under ten cycles of loading.

Materials and Methods

Fifteen tendons taken from the lower limbs from fully mature domestic pigs, weighting approximately 100 [kg] were used in this investigation. Tendons were frozen at -18 ± 2 [°C] until tensile testing, and then thawed in the room temperature one hour prior the experiment. The average diameter was 6,4 ± 0,9 [mm]. Repeated ten loading-unloading cycles were made for three levels of load: 50 [N], 100 [N] and 150 [N]. The test was made with the use of MTS Insight 50 testing machine at a constant rate of strain 5 [mm/min]. Initial sample length was 50 [mm]. Three hysteresis loops for each level of load were registered (1^{st}, 5^{th}, 10^{th}) and used for calculation value of dissipated energy (the area of loop), total work performed on the tendon during stretching and mechanical hysteresis (dissipated energy/total energy) in each loading cycle. Two samples for each load level were used. The calculated values of energies were shown as the average values with a standard deviation (X ± SD).

Results and Discussion

In FIG. 1, hysteresis loops for porcine tendons for three levels of load were shown. For all load levels, the hysteresis is significant over the first loading cycle, but decays quickly after this cycle and between fifth and tenth cycle become nearly steady what correspond to the preconditioned sate of the tendon.
The value of dissipated energy was the highest in the first cycle of loading-unloading (TABLE 1). The value of dissipated energy in fifth cycle was lower by 72, 74 and 77% for the load level 50, 100 and 150 [N] respectively.

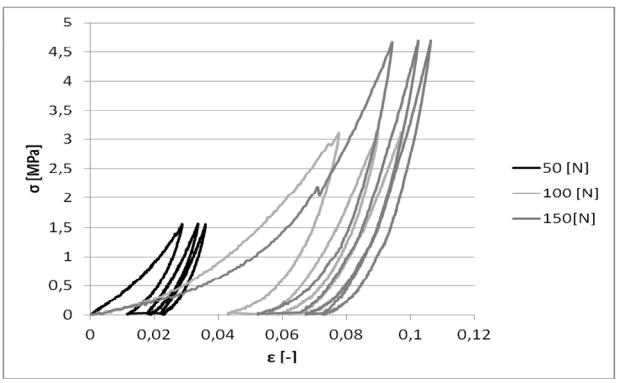

FIG. 1. Hysteresis loops for porcine tendons in ten cycles of loading-unloading.

TABLE 1. Comparision of specific energies calculated for porcine tendons.

Level of load [N]	Energy stored (dissipated) [mJ]	Energy returned [mJ]	Mechanical hysteresis [%]
50	17,40 ± 3,17	14,12 ± 2,80	55,2 ± 1,3
	4,86 ± 0,44	11,70 ± 0,06	29,3 ± 0,2
	3,92 ± 0,33	11,88 ± 0,72	24,8 ± 2,2
100	86,27 ± 2,99	49,93 ± 1,99	63,3 ± 1,5
	22,36 ± 0,54	46,24 ± 1,34	32,6 ± 0,4
	17,08 ± 0,63	44,24 ± 0,89	27,8 ± 0,7
150	117,57 ±3,67	83,52 ± 2,24	58,5 ± 1,6
	26,64 ± 0,80	77,30 ± 1,80	25,6 ± 0,4
	21,18 ± 0,63	75,19 ± 0,97	22,0 ± 0,6

The values of mechanical hysteresis were between 22 and 32% in fifth and tenth cycle (TABLE 1), where stabilization of the dissipated energy was observed. This is in good agreement with hysteresis values given by Maganaris et al. [2] for isolated human tendons in the range between 3 and 38% in tensile testing *in vitro* and between 5-25% in testes *in vivo*. Finni et al. [1] based on literature review, reported that the hysteresis value was between 5-19% for selected animals tendons and between 5-40% for human tendons tested *in vivo*.
A permanent strain set existed immediately after the first loading cycle. The residual strain after first cycle of test was 0,027/0,077/0,094 for the load levels 50, 100 and 150 [N] respectively (FIG. 2). After tenth cycle it was 0,035/0,097/0,106 for the load levels 50, 100 and 150 [N] respectively. The increase in the value of residual strain results from the viscoelastic nature of tendon.

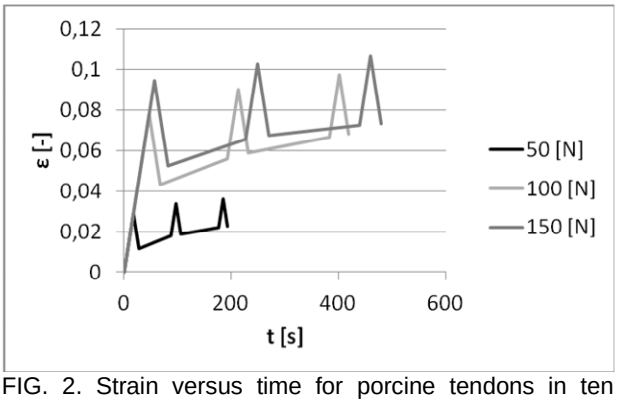

FIG. 2. Strain versus time for porcine tendons in ten cycles of loading-unloading.

Conclusions

The investigation showed that hysteresis of porcine tendon is in the range of hysteresis values reported in the literature [1,2]. The differences in reported hysteresis values are quite large due to many factors. The most significant factors are inter study methodological differences (tendon gripping, cross-sectional area), anatomical site and ageing [2].

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NANOCOMPOSITE MEMBRANES OBTAINED BY A HOT PRESSING METHOD

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[ENGINEERING OF BIOMATERIALS 138 (2016) 56]

Introduction

Bone defects are still a problem in orthopaedics and stomatology. A critical size of bone gaps required some biomaterials which can stimulate process of regeneration of suitable tissues [1]. This membrane implant should show such features as: porosity, bioactivity, defined degradation time and should be handy for surgeons. Commercial polymer membranes are characterized by weak osteoconductivity and osteoinductivity so they work as an inert barrier [2]. Porous polymer system can be functionalized by inorganic, organic and hybrid layers which can create new possibilities in membrane [1-3]. For example, modification of a polymer matrix by such particles as; BG, HAp, TCP lead to bioactive membrane [4]. The presence of MMT particles shortens degradation time comparing to the pure polymer. If the surface is covered by PRP or biological factors its biocompatibility will be higher than in a pure membrane. When the layer consists of biopolymer such as HA it is more suitable for cartilage tissue [2].
The work presents result of investigations on polymer-ceramic nanocomposite membranes which were obtained by hot pressing of porous granules of PLDLA. The polymer matrix was modified by ostoeconductive (SiO_2) and osteoinductive (TCP) particles.

Materials and Methods

Porous nanocomposite membrane based on resorbable poly-L/DL-lactide (PURASORB 80/20, PURAC) granules and ceramic nanoparticles; silica - SiO_2 (5-10 nm, Sigma Aldrich) and tricalcium phosphate - TCP (20-30 nm, Sigma Aldrich) were obtained by a hot pressing method. Porous granules (polymer and nanocomposite) were manufactured by salt leaching method ($NaH_2PO_4 \cdot 2H_2O$, Avator). Given portions of the polymer granules were distributed onto a glass holder and then heated up to 180^{o}C and pressed under 0.6 kPa. Pore size distribution was determined by the mercury porosimetry (PoreMaster 60, Quantachrome Instruments). Microstructure of the nanocomposites was investigated using SEM/EDS (Nova NanoSEM). Thermal properties of the materials after the hot pressing and after nanoadditives were assessed by DSC thermal analysis. Bioactivity test were performed during immersion in SBF medium/7-14 days/37^{o}C. Chemical and morfological changes were observed using SEM/EDS method and FTIR-ATR spectra (BIO-RAD FTS60V). Adhesion and cell spreading were observed in a fluorescence microscope (Olympus CX42) after acrylic orange dyeing.

Results and Discussion

All of manufactured membranes (polymer and nanocomposite) had porous microstructures. Size and shape of the pores strongly depended on salt used as a porogene during the granules preparation. The porosity of granules was independent of the nanofillers i.e.; SiO_2 or TCP. All membranes had similar thickness of 480 µm. Size of the pores present in the membranes were in the range 5-250 µm (FIG. 1). The highest porosity was observed in a pure polymer membrane (PLDLA) then in the membranes modified by SiO_2 and TCP (FIG. 2). The nanoparticles strongly influenced structure of the polymer matrix i.e.; crystallinity of the polymer decreased (DSC) and new bonds were observed in its FTIR spectrum. Both nanocomposite membranes were bioactive: after 7 days in SBF solution on their surface an apatite structure can be observed (SEM/EDS). The apatite structure was observed after 14 and 7 days of incubation in SBF solution on the membrane with n-SiO_2 and TCP, respectively. The biological studies showed that cells (NHOst) preferred spreading near pores. In all porous materials the cells well characterized by proper morphology after 7 days.

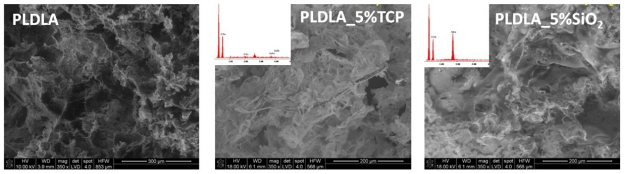

FIG. 1. Morphology of pure polymer, and PLDLA/TCP and PLDLA/SiO_2 nanocomposite membranes.

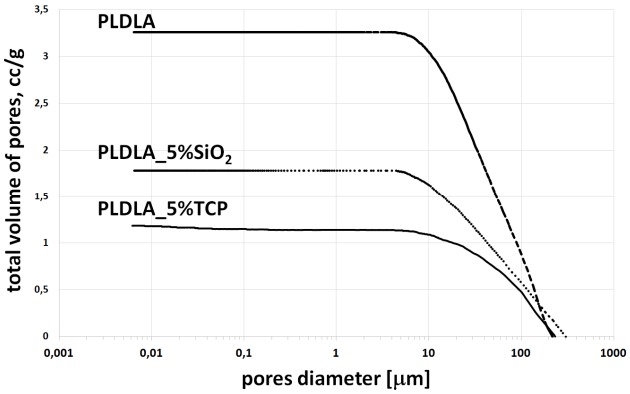

FIG. 2. Pore size distribution of the membranes.

Conclusions

Hot pressing method is a simple and cheap way to produce membrane materials. It guarantees repeatability of materials with similar weight, size of pores and total porosity. Nanocomposite membrane materials based on PLDLA and modified with SiO_2 or TCP give possibility to design such material features as; bioactivity and biocompatibility. It means that nanocomposite membranes can be potential materials for space making implants in a bone tissue defect.

Acknowledgments

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CARBON NANOCOMPOSITE MEMBRANE WITH BIOACTIVE FILLERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 57]

Introduction

Carbon fibers are widely used in medical applications. All types of carbon materials (micrometric fibers, carbon nanotubes, graphene) show properties that are important for different kinds of implants. Some carbon materials may be applied on their own (CNT), while others are used to modify a polymer matrix (and used as composites, nanocomposites).

Electrospinning is a new technique to obtain fibers with a polymer characterized by submicrometric and nanometric diameter. The most important asset of such biomimetic fibers is their size and shape that imitate human tissues (ECM, collagen).

It seems purposeful to design a fibrous system conducive to bone regeneration. In the literature it is proved that carbon fibers obtained from polymer precursor (polyacrylonitrile, PAN) and then thermal treatment. Such fibers may be modified during a spinning process by adding bioactive nanoparticles (e.g. tricalcium phosphate, hydroxyapatite, bioglass or silica). Studies of similar - yet micrometric - compositions displayed their bioactive properties.

In the present work the electrospinning method was used to obtain nanocomposite fibers PAN/TCP and PAN/SiO_2 composed of nanometric and submicron fibers enriched with TCP or SiO_2 respectively. The fibers underwent two-stage thermal treatment: oxidation and carbonization. During these processes the structure of the base polymer changed. As a result, nanocomposite carbon fibers were produced (CNF/TCP and CNF/SiO_2). Morphological analysis of the fibers was conducted at every stage of the treatment. The properties of the final carbon substrate, including its bioactivity, were also assessed.

Materials and Methods

Commercially-available polyacrylonitrile (Sigma Aldrich) was used in the study (molecular weight 150 kDa, density 1.18 g/ml at 25^{o}C). DMF (CZDA, Avantor) was used as a solvent. 12% polymer solutions were prepared and nano additives TCP and SiO_2 (5% wt) were dispersed in the solutions. Nanometric particles had the following sizes: SiO_2 5-10 nm (Sigma Aldrich), TCP 30-50 nm (Sigma Aldrich). The compositions were homogenized mechanically and then sonified before inserting them into the electrospinning machine. The fibers were obtained on the drum collector at 12 kV and 32^{o}C. The oxidation process lasted for 30 minutes at 250^{o}C. The carbonization took place in protective atmosphere (nitrogen) and it had two stages: 720^{o}C/30min and 950^{o}C/30min. The morphology of carbon fibrous membrane was established by means of SEM (Nova NanoSEM), while the presence of fillers (TCP, SiO_2) - using EDS analysis (Genesis). The diameters of nanocomposite fibers and their contraction after the thermal treatment were assessed, too. The nanocomposite membranes were incubated in simulated body fluid (SBF) for 7 and 14 days (37^{o}C/5%CO_2) to run the bioactivity test. The SEM/EDS analysis confirmed bioactivity of the materials and the apatite growth on their surface. The structural tests were also conducted to establish the composition of the layer on the surface of the membranes (FTIR-ATR).

Results and Discussion

The polymer fibers with PAN measure approx. 350 nm in diameter. The addition of modifiers causes the diameter growth to 380 nm for TCP and 360 nm for SiO_2 respectively. Thermal treatment (oxidation) results in obtaining the fibers of 280 nm in diameter for PAN, 290 nm for PAN/TCP and 250 nm for PAN/SiO_2. In turn, carbonation influences the contraction growth of membranes: 150 nm (PAN), 180 nm (PAN/TCP) and 150 nm (PAN/SiO_2). EDS analysis indicates the presence of elements attributed to nanofillers: silicon for SiO_2, calcium and phosphorus for TCP (FIG. 1). However, the number of these elements is minimal. The PAN/SiO_2 membrane seems to be a more homogeneous composition. It is probably related to a smaller size of its particles (SiO_2: 5-10 nm). The incubation in SBF confirms the apatite growth on the CNF/TCP membrane after 7 days, whereas on the CNF/SiO_2 membrane it appears after 14 days. After that period of time on the CNF/TCP membrane no traces of fibrous substrate are visible. There is no apatite on the reference substrate of pure CNF.

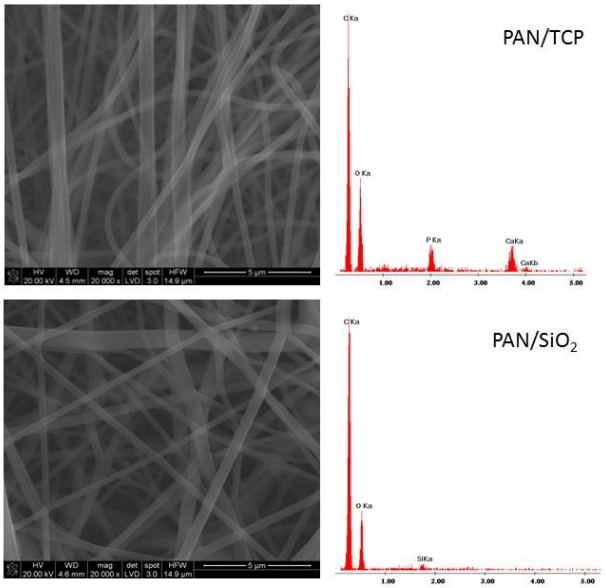

FIG. 1. Microstructure of polymer nanofiber (SEM/EDS) with PAN/TCP and PAN/SiO_2.

Conclusions

It was demonstrated that the electrospinning method makes it possible to obtain polymer nanocomposite membranes modified with TCP and SiO_2 respectively. Having undergone the thermal treatment, such fibers change into carbon fibers that are still nanocomposites: CNF/TCP and CNF/SiO_2. Both kinds of fibers display bioactivity in *in vitro* conditions (SBF).

Acknowledgments

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ELECTRON WORK FUNCTION AS A DIRECT PARAMETER FOR BACTERIAL INFECTION RISK OF IMPLANT SURFACES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 58]

Introduction

The risk of biomaterial centred infection (BCI) is the main drawback limiting their use. To prevent bacteria colonization of the metal implants surface, several approaches which require precise engineering of their surface architecture and properties can be considered e.g. changing surface chemistry and functional groups and/or introducing topographical features on the surface (micro– and nanopores). All of the mentioned practises aim in favouring osseointegration over hazardous bacteria attack [1]. Since the bacteria exhibit the charge on the surface (partial charges accumulated on wall teichoic acids functional groups) it is expected that the electrostatic potential between implant surface and bacteria can play a crucial role in their adhesion.

The aim of the study was to evaluate if there is any correlation between electrodonor properties of the implant surface and bacterial adhesion. To address this problem, a series of implant material were prepared with the same chemical and structural composition while their nanotopography and thus, the work function values, varied. The second stage of the study involved evaluation of the prepared surfaces for bacteria adhesion.

Materials and Methods

Nanoporous anodic titanium oxide (ATO) layers were prepared by three-step anodization in a standard two-electrode electrochemical cell with a platinum plate as the cathode and the titanium foil as the anode. An ATO layer formed on a Ti substrate has several advantages like nontoxicity, biocompatibility and osseointegration. The anodization experiments were carried out at a constant potential of 30-100V in an ethylene glycol with NH_4F and H_2O [2].

The contact potential difference (V_{CPD}) measurements were carried out by the Kelvin method with KP6500 (McAllister Technical Services). For bacteria adhesion test *S. aureus* 24167 DSM with net negative surface charge was selected.

Microbiological tests were performed for three independent series in triplicates according to the procedure described elsewhere [3]. Area occupied by *S. aureus* was determined with the use of fluorescence microscopy. Prior observations, bacteria cells were fixed and stained with propidium iodide.

Results and Discussion

The series of ATO samples differed substantially in terms of surface electronic properties gauged by the work function. The highest work function was found to be 4.23 eV for the samples anodized at 30 V, whereas the lowest value of 3.98 eV for 100 V. For various anodization potentials the samples exhibit different pores diameters and also the titania wall thickness. As a result, work function changes in non-linear way. Such changes substantially modify the bacteria adhesion to the titania surfaces. The adhesion was quantified by the area occupied by bacteria on the investigated ATO surfaces. The profile exactly follows the non-linear trend observed for work function measurements with the highest occupied area of 12%, observed for the highest work function values. The general revealed trend is schematically presented in FIG. 1.

The obtained results can be interpreted in terms of microbial adhesion to implant surfaces as primarily mediated by non–specific interaction forces which include Lifshitz–Van der Waals forces and electrostatic forces, which both operate over a long range, as well as hydrophobic and acid-base interactions that act over a shorter range. After approaching the implant surface, microorganisms are attracted or repelled by the biomaterial surface, depending on the resultant interaction forces. It should also be noticed, that this situation takes place immediately after implant placement in the environment of body fluids before the surface is conditioned (adsorption of proteins and peptides). This stage is critical in the 'race for the surface' between bacteria and osteoblasts and decisive for the appropriate host response after surgery. Winning the competition between bacteria and osteoblasts is the ultimate goal of surface implant engineering.

The obtained results clearly indicate the infection-reducing strategy based on the concept of implant surface work function lowering. Since the repulsion electrostatic forces between surface and bacteria play a key role in the weakening of irreversible adhesion, which is an initial step in biofilm formation, favouring osseointegration.

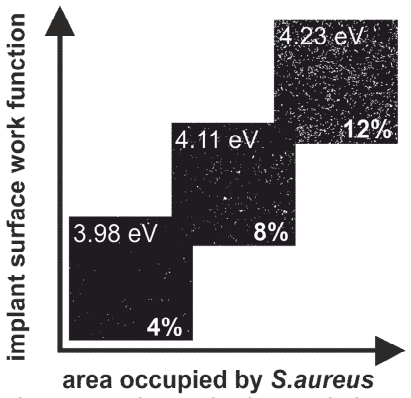

FIG. 1. The general trend observed for *S. aureus* adhesion to titania implant surfaces.

Conclusions

Strong correlation between *S. aureus* adhesion on the ATO surfaces and the electron work function of this implantable material was discovered. The conducted study proved that the work function can be applied as a direct parameter for evaluation of surfaces against bacteria adhesion.

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AN INTEGRAL APPROACH FOR TAILORING OF IMPLANT-TISSUE INTERFACE: PLGA-PARYLENE C MULTIFUNCTIONAL COATING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 59]

Introduction

The challenges of metal implant engineering are to introduce specific properties which result in optimization of the metal-implant tissue interface. Among various approaches, one of the most explored is coating metal implants with a polymer which can be additionally provided with functions essential for a long-term implantation success, namely: anti-corrosive, biocompatibility, anti-infection and therapeutic.

Extensive research worldwide is carried out on improving biocompatibility of implant surfaces in terms of osteoblasts adhesion and anti-bacterial function. Furthermore, the most common post-operation complications include prolonged inflammation and biomaterial-centered infection. For these reasons, main strategies rely on surface functionalization and/or controlled local drug release from the inserted implant, bringing a number of benefits to patients. The most significant advantages include the target tissue, which reduce the risk of side effects associated with infection as well as oral administration of high doses of medication.

The aim of the study was to develop multifunctional polymer coating based on drug+PLGA/parylene C with four essential functions. The general concept of integrated research is highlighted.

Materials and Methods

Parylene C films were prepared by CVD technique. To generate oxygen-containing functional groups and nanotopography, polymeric samples were modified using oxygen plasma with parameters which enhance its biocompatibility, remaining the bulk properties intact.

For the therapeutic layer preparation, the biodegradable D,L-lactide-co-glycolide copolymer (PLGA) (85/15) was used [1]. PLGA was dissolved in CH_2Cl_2 with ibuprofen or gentamicin and deposited on oxygen plasma modified parylene C with the use of airbrush method. The drug+PLGA/parylene C coating was then thoroughly characterized using surface-dedicated (SEM, μFTIR, LDI-MS) and biological (*in vitro* cells and microbiological tests). In order to identify drugs elution kinetics, *in vitro* drug release studies were carried out. The release data were fitted into kinetic models (first order and Korsmeyer-Peppas) [2].

Results and Discussion

The key functions of the designed coating are the anti-corrosive properties, biocompatibility, anti-infection and therapeutic (FIG. 1). Parylene C micrometric coatings provided superior increase in corrosion resistance (1×10^9 Ω cm^2) when compared with uncoated SS 316L (1×10^4 Ω cm^2) [3]. Surface modification of parylene C caused changes in its chemical composition by generation of functional groups such as –COOH, –OH as well as nanotopography. Fluorescent staining of focal contacts of MG-63 cells together with SEM observations revealed improved biocompatibility of oxygen plasma modified parylene C. The area of focal contacts (FC) was quantified for oxygen plasma treated samples and compared to unmodified parylene C, where the FC level was minor or below the detection limit. The average area of FC was 6.21±1.2 μm^2 which is comparable with the contact area created by cells in the control well of TCP (6.53±1.6 μm^2).

Generated nanotopography, effectively limited the surface area available for bacteria. SEM observations revealed, that early-stage biofilm formation on unmodified parylene C takes place after 4 h of incubation, after the same time interval, on the surface of oxygen plasma treated samples not agglomerated single bacteria cells dominated the picture.

The studies of drug+PLGA/parylene C systems revealed that the drugs molecules remain unchanged upon interaction with the PLGA matrix and the drugs distribution were homogenous. The obtained release profiles revealed that both of the investigated systems (ibuprofen- and gentamicin-loaded) are suitable for prolonged elution up to 21 days. However, they follow different kinetic models. For ibuprofen+PLGA/parylene C samples, the average drug load was 180 μg/cm^2. The drug elution was governed by dispersion and diffusion with non-Fickian transport mechanism. The antibiotic release from gentamicin+PLGA/parylene C was diffusion dominated (quasi Fickian drug migration through porous PLGA matrix), with average drug load 1.5 μg/cm^2.

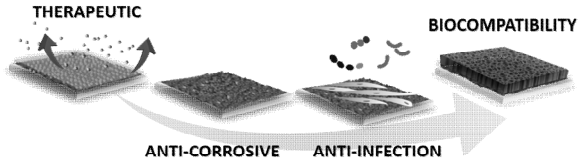

FIG. 1. The overview of the conducted research strategy based on four key functions essential for implant long-term success.

Conclusions

Oxygen insertion into the parylene C surface provides a suitable substrate for MG-63 cells attachment and spread while nanoroughness effectively limits risk of infection. Modified parylene C allows also further tuning of the coating functionality by bonding of a biodegradable drug–loaded PLGA results in prolonged in-site drug release up to targeted 21 days.

Acknowledgments

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EFFECTS OF CARBOXYLIC ACID ADDITION ON THE SETTING TIME AND COMPRESSIVE STRENGTH OF GLASS-IONOMER CEMENTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 60]

Introduction

Glass-ionomer cements are created through the curing process of compositions made of two components: glass powder and a bonding liquid — an aqueous solution of polyalkenoic acids. Cement setting is based on an acid-base reaction; when ions of metal elements released from the glass surface bond with polyanions derived from the polyacid. Since the reaction is very fast, various compounds are added to the bonding liquids to extend the composition setting time. These are most often carboxylic hydroxyacids capable of complexing ions released from the glass [1-3]. Moreover the kinetics of the glass-ionomer cement curing process may also depends on other factors like pH or structure [4,5].

Materials and Methods

For the purposes of the study, the following components were used:
- G-J CHEMADENT powder (calcium-fluoroalumino-silicate glass) and G-J CHEMADENT liquid (aqueous solutions of acrylic and itaconic acid (AA-IA) copolymer) synthesised in accordance with the patent description "Dental glass-ionomer composition" [6] as cement components for dental fillings and restoration in the Department of Ceramic Technology of ICiMB;
- carboxylic hydroxyacids: tartaric acid (Avantor), citric acid (Avantor), malic acid (Fluka), and dicarboxylic acid
- oxalic acid (Avantor).

Bonding liquids were obtained on the basis of the solution of AA-IA copolymer, water and the above listed carboxylic acids. Glass-ionomer composition samples were made on the basis of powder component and the obtained bonding liquids in quantitative ratio 2,4g:1ml, in the way and conditions determined by a standard PN-EN ISO 9917-1. Properties of the glass-ionomer compositions were determined through the setting time measurement, while the properties of the cured cements were determined on the basis of compressive strength.

Results and Discussion

The addition of carboxylic hydroxyacids to AA-IA copolymer solution caused reduction of their viscosity and had positive influence on the mixing process of the glass-ionomer compositions through reduction of setting time. The highest increase in the composition setting time was observed for the addition of malic acid to the bonding liquids (FIG. 1).

The addition of carboxylic hydroxyacids to bonding liquids had negative influence on the strength of the cements obtained (FIG. 2). Test results showed that cements containing bonding liquids with added malic acid of 5% and 10% demonstrated the highest compressive strength, but higher content of this acid caused the higher reduction in cement strength to values below 100 MPa, which does not meet the requirements of the above mentioned standard.

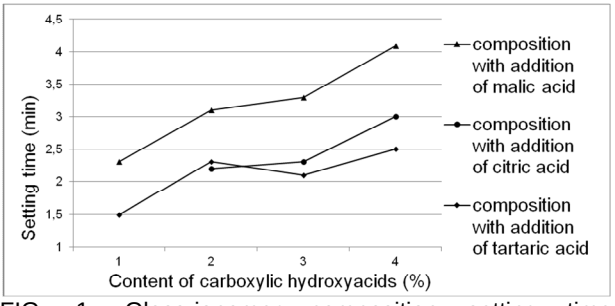

FIG. 1. Glass-ionomer composition setting time depending on the type and content of carboxylic hydroxyacids.

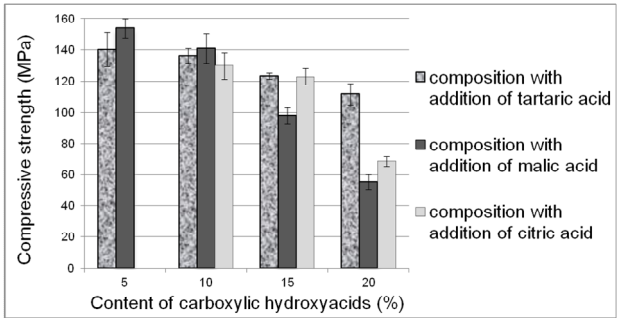

FIG. 2. Glass-ionomer cement compressive strengths depending on the type and content of carboxylic hydroxyacids.

It is known from the literature, that oxalic acid may function in glass-ionomer compositions as a setting reaction modifier [5]. Tests of compositions containing oxalic acid demonstrated that small additions of this acid (0,5 wt% and 1 wt%) affect the most setting time values. Depending on the type of additives and their percentage content in the bonding liquid, the composition setting time may be reduced or extended.

Strength test results of cements based on bonding liquids containing oxalic acid don't demonstrate a considerable influence of oxalic acid on the compressive strength of cements that contain it.

Conclusions

Test results indicate that the addition of carboxylic hydroxyacids, especially malic acid, reduces the bonding liquid viscosity, extends the glass-ionomer composition setting time, and facilitates cement mixing, but unfortunately reduces their compressive strength. The addition of oxalic acid to the bonding liquid may modify the setting time of glass-ionomer composition, without changing the strength properties of the cements.

Test results indicate that there may be several factors that simultaneously affect the glass-ionomer composition setting process, which may be of significance in developing materials for new applications.

Acknowledgments

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GENOTOXICITY OF ANTIBACTERIAL BIOGLASSES OBTAINED BY SOL-GEL METHOD FOR *SALMONELLA TYPHIMURIUM*

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[ENGINEERING OF BIOMATERIALS 138 (2016) 61]

Introduction

Periodontal disease causes problems in dentistry. Surgical intervention with appropriate biomaterials for tissue regeneration is necessary in advanced stages of the disease. Due to the risk of bacterial contamination during the regeneration of bone tissue in the oral cavity, studies are continually being undertaken with a view to creating new or modifying existing biomaterials and providing them with bactericidal properties [1,2].

Some of the materials that could potentially have medical uses contain substances exhibiting various types of toxicity. One of them is genotoxicity. It consists in causing the mutations – permanent, inheritable changes in hereditary substance (deoxyribonucleic acid - DNA). Some mutations can induce cancer, namely carcinogenesis. Therefore, before the introduction of new medical materials for widespread applications, they are examined to determine the genotoxic properties. The Ames test is one of the many bioassays for identifying the mutagenic activity of tested specimens [3,4].

Therefore, the aim of this study was to determine the mutagenicity of B-I calciumsilicate as well as Z-5 and Z-8 aluminosilicate bioglasses for *Salmonella typhimurium* in the Ames test.

Materials and Methods

The study involved bioglasses (TABLE 1) obtained by the sol-gel method using tetraethyl orthosilicate substrates as a silica precursor and aluminum isopropoxide, nitrate tetrahydrate calcium, triethyl phosphate and silver nitrate. The physicochemical properties e.g. grain morphology and the cytotoxicity and antibacterial potency of these bioglasses are known from earlier reports [5-7].

TABLE 1. The oxide compositions of tested bioglasses.

bioglass	content, wt %				
	SiO_2	Al_2O_3	CaO	P_2O_5	Ag_2O
Z-5	95,7	0,8	-	-	3,5
Z-8	89,0	7,5	-	-	3,5
B-I	60,0	-	37,0	2,0	1,0

Extracts of bioglasses were introduced into the test as solutions in DMSO. They were partially diluted to obtain B-I and Z-8 bioglass doses of (0,25, 0,5, 1, 2, 4, 8) mg/cm^3 of the mixture during exposure and a Z-5 bioglass dose of (0,125, 0,25, 0,5, 1, 2, 4, 8) mg/cm^3. The test bacteria was exposed to six dilutions of test samples for 90 minutes in a 24-well microplate in three replicates for each dilution.

The genotoxicity of bioglasses were provided by the Ames Xenometrix by Endotell microplate test using TA 98 and TA 100 *Salmonella typhimurium* strains (Ames MPF^{TM} 98/100). Tests were carried out with and without metabolic activation of 30% rat liver S9 microsomal fraction. Tests were performed according to the procedure described in the manufacturer's instructions; the result was positive when the number of holes containing revertants was at least three times greater than the negative control.

An Excel spreadsheet provided by the manufacturer of the test was used for statistical analysis. The statistical significance of the differences in the number of revertants between test samples and negative controls were studied in a unilateral t-test and were considered significant at $p \leq 0.05$. According to the procedure, the test results were considered reliable because the average number of positive holes (from the revertant) did not exceed 8 in the negative control for a TA 98 and 12 for the *Salmonella typhimurium* strain, and did not exceed 25 for the TA 100 *Salmonella typhimurium* strain in the positive control.

Results and Discussion

B-I calciumsilicate bioglass showed mutagenic activity against the TA100 strain with metabolic activation of the S9 fraction, and did not demonstrate mutagenic activity against the TA100 strain without metabolic activation or the TA98 strain with and without metabolic activation. They did not include direct mutagens causing base-substitution mutations and direct and indirect mutagens caused frame-shift mutations, the detection of which allows the TA98 strain. In the B-I bioglass could remain traces of substrates used to make it. Some of them cause the formation of different mutations [8-12]. The collected literature data indicate that reversion of mutations in the TA 100 strain in the presence of S9 caused by B-I bioglass could be a consequence of the combined effect of its components and the substrates remains.

Z-5 and Z-8 aluminosilicate bioglasses did not exhibit mutagenic activity applied to the *Salmonella typhimurium* strains tests with and without metabolic activation of S9 fraction in the tested concentrations.

Conclusions

The results indicated that the presence of intermediate mutagens in the B-I calciumsilicate bioglass cause base-substitution mutations.

The Z-5 and Z-8 aluminosilicate bioglasses did not exhibit mutagenic activity against TA 98 and TA 100 *Salmonella typhimurium* in the Ames test, with and without metabolic activation. This means that they do not cause frame-shift and base substitution mutations, which the applied strain allows for detection. Thus, there should be further study of Z-5 and Z-8 bioglasses prior to their clinical application.

Acknowledgments

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CANDIDACIDAL ACTIVITY OF SALIVA PREPARATION CONTAINING GOLD NANOPARTICLES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 62]

Introduction

The oral cavity is a specific ecosystem, where important function plays secretion of salivary glands called saliva. Natural saliva contains antibacterial factors and is characterized by specific protective mechanisms beneficial for oral balance. It provides optimal pH, which is dependent on electrolytes and buffer systems like phosphates, proteins and bicarbonates, protecting teeth against enamel demineralization [1]. However, in some pathological situations (depression, cancer treatment, medicaments ingestion), a large number of people suffer from impaired salivary functions, displaying symptoms of xerostomia. This process is characterized by a reduction or loss in salivary flow, often with a concurrent change in the composition of the saliva, resulting in dryness in the mouth. Disorders of human saliva secretion may lead to many pathological changes in oral cavity. One of them is candidosis of oral cavity mucous membrane, caused by *Candida albicans*.

Thus, there is a demand from patients for more effective and simultaneously safe products, which assist the normal processes occurred in oral cavity and give protection against sickness microorganisms. The saliva preparations available on the pharmaceutical market are characterized by different composition, texture, biological and chemical properties. However there is a lack of preparations which fulfil the requirements demanded of them [2]. This particularly pertains to new synthetic saliva additives that will reduce the growth of microbes and still will have an advantageous influence on the rheological properties of synthetic saliva compositions. In the context of such antimicrobial compounds, gold and silver are of great interest, particularly in form of nanoparticles. Additionally, as noble metals, they are not corrosive, which is of great significance when consider their contact with metal prostheses in oral cavity.

The main goal of this work was to prepare gold nanoparticles based saliva substitutes, which rheological and tribological properties are similar to natural saliva and were evaluated in previous works [3]. The utilitarian effect of this work is an evaluation of the influence of gold nanoparticles on *Candida albicans* cells adhesion to surface of biomaterial used in prosthetic dentistry.

Materials and Methods

Biological analysis was performed using *Candida albicans* (PCM 1407). Tests of *Candida albicans* microorganisms adhesion to surface of widely used in stomatology and prosthetic dentistry material (Co-Cr-Mo alloy) with rough and smooth surfaces were tested in the present study. Chemical composition of tested solutions is presented in TABLE 1.

TABLE 1. Chemical composition of tested solutions.

Environment	Chemical composition
Control	nutrient medium
A	PBS + mucin + xanthan gum
B	PBS + mucin + xanthan gum + gold nanoparticles

Results and Discussion

The microscopic observations results performed using confocal scanning laser microscopy (CSLM) shown that *Candida* cells adhered especially to rough surfaces.

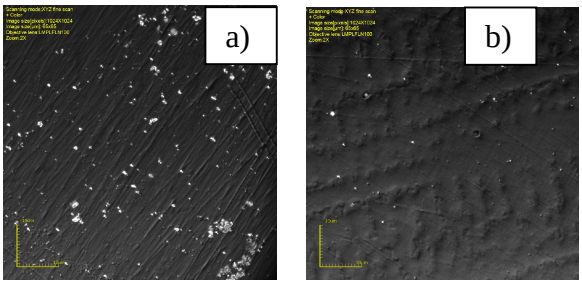

FIG. 1. CSLM micrographs of Co-Cr-Mo alloy after contact with nutrient medium (a) and preparation B (bght picture), bars, 10 μm.

Obtained results shown that *Candida albicans* adhesion on metal surfaces was observed in the case of control (nutrient medium) at the highest extent (FIG. 1a) Numerous single and colonies of *Candida* cells with gemmate signals were observed at tested surfaces. Similar results, although less intensive were obtained for cobalt alloy incubated in saliva preparation without addition of gold nanoparticles. In FIG. 1b is shown biomaterial surface after incubation in saliva substitute with addition of gold nanoparticles. It can be concluded that this preparation inhibited *Candida albicans* growth on CoCrMo alloy surface.

Conclusions

In all tests it was observed that microbes adhesion was more intensive to rough surface in comparison to smooth one. Saliva preparation with gold nanoparticles addition inhibits growth of C*andida albicans* cells on tested biomaterial surface. These results confirmed that gold nanoparticles exhibit antifungal properties.

Acknowledgments

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APPROACH TO SELECTION OF STERILIZATION METHOD FOR BIODEGRADABLE POLYMERIC MEDICAL DEVICE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 63]

Introduction

Designing of a new medical device, besides such indispensable factors as shape configuration, dimensions, selection of biomaterials, involved manufacturing processes, should also encompass a potential sterilization method. Scientists, especially in academia, involved in early stage development often underestimate the possible detrimental effect of sterilization on material properties, although sterilization is critically needed when it comes to commercialization of the new device.

Validation of chosen sterilization method in terms of its efficacy, reliability and reproducibility is one of the requirements the manufacturer is requested to demonstrate to the notifying or certifying authorities, and its account is included in a 'technical file' of a new device. In order to fulfil the obligations, 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 with regards to sterilization, and further, the guidance of ISO standards. Those standards address general requirements (e.g. EN 556) and provide recommendations to the most common sterilization methods.

Those applicable for medical devices encompass moist heat, formaldehyde, ethylene oxide, radiation and plasma. Particularly, implants based on biodegradable synthetic polymers require special concern with regards to their sterilization. In principle, only low-temperature methods are acceptable. Simple implants, designed for load bearing applications, i.e. of polylactides, are typically treated with EO. Other chemical methods should be avoided since sterilizing agents may interact with polymers in terms of chemical reactions, or modify greatly its surface chemistry, e.g. hydrogen peroxide plasma. Moreover, polymeric biomaterials are not inert to (high energy) radiation. Irradiation of a polymer generates radicals, precursors of reaction leading either to crosslinking or degradation. The latter case is usually detrimental to polymer physical properties, thus a number of polymers cannot withstand radiation sterilization. Polyesters degrade when exposed to ionizing radiation, yet those possessing in their chemical structures secondary carbon atoms may simultaneously undergo crosslinking. A good example is poly(ε-caprolactone) (PCL) in which radiation causes broadening of molecular weight but ultimately, at higher doses, a gel is formed.

A short review of regulatory requirements will be presented, and followed by a case study of sterilization approach applied for a biodegradable medical device comprising a polymer with supramolecular chemistry based on ureidopyrimidinone (UPy) (FIG. 1). Implantable electrospun mesh is intended to be used for reinforcement and repair of soft tissue, e.g. operational treatment of pelvic organ prolapse and stress urinary incontinence, frequent disorders in ageing women.

Materials and Methods

Ureidopyrimidinone modified PCL was synthesized based on know-how of SupraPolix [1]. Electrospinning system of Coloplast was applied in order to fabricate nonwoven mesh. Various methods of sterilization were challenged, such as ethylene oxide (EO), hydrogen peroxide plasma, hot steam, electron beam (EB) and gamma radiations. Tensile testing of the implant and molecular weight changes of the UPy-PCL were followed by cytotoxicity testing with LDH and XTT assays.

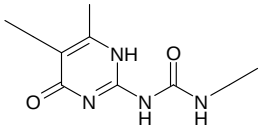

FIG. 1. Ureidopyrimidinone moiety incorporated into PCL chain.

Results and Discussion

Since UPy-PCL is sensitive to elevated temperature, and autoclaving melted the polymer, only low-temperature methods were further considered for sterilization of this biomaterial and consecutive implants. Sterilization with EO resulted in partial deformation of the implant and fusion of the mesh fibers – the temperature of the process, i.e. even up to 60^{o}C is too high. Sterilisation with low temperature plasma did not deformed the implant, yet the method was considered to be not full reliable since one cannot be assured that entire high density mesh with porosity of c.a. 10 μm is effectively penetrated with the ionised gas, therefore it was impossible to demonstrate microbiological cleanness of the implant.

Studies on radiation sterilization of the implant by either electron beam and gamma rays showed suitability of the radiation method. Changes in mechanical properties of the mesh caused by irradiation with 25 kGy were minor, and resulted from small reduction in molecular weight of the polymer (c.a. 10% for EB). Gamma irradiation had somewhat greater negative impact on the material performance. Significance of dose rate was proved. Validation of radiation method based on ISO11137 of VD_{max} approach was conducted [2].

Quantitative cytotoxicity tests of radiation sterilised UPy-PCL mesh implant showed that the material does not induced detrimental effect towards cells, what demonstrated that radiation sterilization does not alter biological safety of the material.

Conclusions

Selection of potential sterilization methods, their comprehensive review at the early stage of implant development should be followed by testing of biomaterials exposed to predetermined most promising sterilization factor, which in turn may be beneficial in further work and cost reduction related to accomplishment of certification requirements. Radiation sterilization of EB was demonstrated to be the most suitable among low temperature sterilization methods towards an implant of e-spun mesh based on supramolecular UPy-PCL.

Acknowledgments

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APPLICATION OF IONIZING RADIATION FOR SYNTHESIS, MODIFICATION & STERILIZATION OF NOVEL BIOMATERIALS AND INNOVATIVE MEDICAL DEVICES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 64]

Introduction

Sterility is one of indispensable requirements for proper performance of biomaterials when utilized in medical device manufacturing. Ionizing radiation is well-established tool to sterilize medical devices and their packaging, especially in relation to their polymeric component. Beside reduction of bioburden (virtually to zero) during sterilization, radiation treatment may cause substantial changes in physicochemical properties of biomaterials. Radiation initiates essential reactions in polymers, such as polymerization, degradation, crosslinking, grafting, oxidation, etc., thus can be utilized to manufacture or modify polymeric biomaterials.

Besides studies on fundamental radiation processes, our research group led by prof. Rosiak, in the last 30 years, developed a number of radiation technologies of polymeric biomaterials [1]. Some of them have been commercialized (e.g. hydrogel wound dressings or hydrogel dosimeter for radiotherapy), some are being further modified by other laboratories and companies in Poland and abroad, while other still await industrial investments (e.g. hydrogel systems for induction of childbirth, hydrogel-based hybrid artificial organs or hydrogel-based dietary products).

Materials and Methods

Ionizing radiation facilities available at Lodz University of Technology can generate electron beams (electron accelerator) or gamma rays (^{60}Co sources), that are used to initiate radical-mediated reactions in polymers. These are water-soluble polymers, of synthetic and natural origin, typically irradiated in aqueous solution, or synthetic biodegradable polyesters, polycarbonates, their blends or copolymers. Studies on sterilization of pioneering medical devices and sterilization validation are conducted according to ISO regulations, especially of 10993 and 11137 series.

Results and Discussion

Macroscopic hydrogels, three-dimensional networks of hydrophilic polymers, found a number of practical applications in the field of biomaterials. Advantage of radiation-induced initiation for hydrogel manufacturing is avoiding any additives, as the intermolecular crosslinking reactions are initiated in a pure polymer-solvent system. [2]. Hydrogels fabricated by radiation technique can be used as 3D constructs for tissue development. For instance, gels formed by irradiation of aqueous solutions of monomers were modified with laminin [3]. Embryonic SC seeded within hydrogel scaffold were differentiated into neurons and further stimulated by microelectrode arrays exhibited neural-like tissue properties of memory acquisition and learning electrical stimulus. This may be used in pharmacological and toxicological applications to mimic neural tissue in order to reduce in vivo experiments.

Method of synthesis of nano- and micro-gels elaborated in our group (first in the world) employs high-dose-rate irradiation of dilute aqueous solution of hydrophilic polymer. Numerous radicals created at each single chain recombine intramolecularly to form crosslinked structures of single or few chains, i.e. nano- and micro-hydrogels. This method successfully competes with classical ways of manufacturing gels for application as drug carriers, chiefly because of their chemical purity [4].

Stimuli-responsive surfaces for cultivation of skin cells for treatment of large burn wounds were synthesized by radiation graft polymerization of a thermoresponsive monomer from regular cell cultivation vessels. Harvested and seeded fibroblasts proliferate to form a monolayer, which can be straightforwardly removed from the surface by reduction of temperature – the cells detach without damage, what greatly improves efficacy of the procedure – and can be transplanted onto the burn wound [5].

Radiation can cause detrimental changes in biomaterials based on biodegradable synthetic polyesters (e.g. PLA), thus incorporation of crosslinking type polycarbonate (PTMC) is beneficial for improving radiation stability of the polyester [6], and also helps adjusting mechanical properties and biodegradation kinetics. Guides for peripheral nerve regeneration require advanced biomaterial engineering, i.e. the three-step technology developed at TUL, which involves 1) manufacturing of guide tubes of PLA and PTMC blend by spraying method, 2) filling the tube with physical gel of selected polysaccharide, and after packing 3) EB irradiation [7]. Irradiation with 25 kGy causes crosslinking of the gel to form internal scaffold in the tube, moreover the implant is ready to use since sterilization is achieved simultaneously.

Sterilization method for novel biodegradable e-spun mesh, based on supramolecular polymers of ureido-pirymidinone moieties in the main-chain of polyester or polycarbonate (UPy-polymer) intended to be applied for chirurgical treatment of pelvic organ prolapse has been selected. EB irradiation is the method that guarantees sterility of entire product (SAL 10^{-6}), and the physical-chemical properties changes are acceptable when the product is packed under moisture-free protective gas.

Conclusions

Sterilization, yet the main application of ionizing radiation in biomedical field, is not the only way to exploit its potential. The other, so called 'radiation engineering of biomaterials', evolved in Poland from the technologies developed at TUL, with its applications become useful, still not widely known branch of radiation processing.

Acknowledgments

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THE INFLUENCE OF THE STERILIZATION PROCESS ON THE MICROSCOPIC STRUCTURE OF HYALURONIC ACID-BASED NANOFIBROUS SCAFFOLDS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 65]

Introduction

Regenerative medicine is one of the fastest growing areas of contemporary medicine. Constant search for newer and newer biomaterials creates the possibility of taking up the close cooperation between scientists from many areas of science, including biology, material science, chemistry and medicine. Currently, one of the groups of polymers that are widely used in biomedical engineering are various types of polysaccharides, such as alginates, chitin and its derivatives and hyaluronic acid. Alginates have been used in regenerative medicine in various types of materials for the regeneration of muscle tissue, scaffolds for recovering the bone and cartilage tissues, as well as dressing materials adapted to various stages of wound healing [1-4]. Materials based on chitin derivatives can be found in many applications in the field of regeneration of skin tissue in the case of difficult-to-treat wounds of different aetiologies. Recently, such polymers have also found applications in bone tissue engineering. In contrast, materials based on hyaluronic acid are used mainly in aesthetic medicine as well as regeneration of skin tissues. The new application of a hyaluronic acid comprising very high biocompatibility can be the use thereof in the preparation of hybrid materials for both - the treatment of tissue defects of skin and bones. Due to its high hydrophilic properties it can be used as a reservoir for biologically active compounds such as medicines, nano- and microadditives, cell growth factors, as well as others.

The paper presents the behaviour of materials based on hyaluronic acid under the influence of the sterilization process. The essence of the problem was based on the observation of microscopic structure of the substrate of hyaluronic acid treated with various types of radiation.

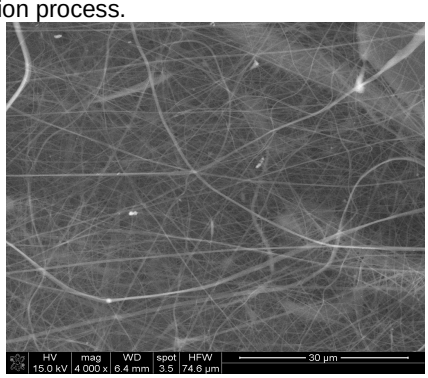
Materials and Methods

For production of nanofibers, the hyaluronic acid sodium salt was used (Contipro Biotech, Czech Republic) with a molecular weight of M=100-150 kDa. The low molecular weight of hyaluronic acid provided the ability to use higher concentrations of the spinning solutions. The analysis of the microscopic structure of the nanofibers was performed based on the scanning electron images obtained by FEI NOVA Nanos 230 equipped with an electron gun with a field emission (FEG).

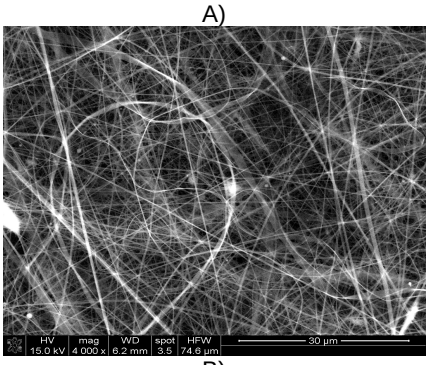
The samples were irradiated with a dose of 25 kGy in air by means of accelerated electrons from the accelerator ELU-6 (Eksma) having a horizontal beam. Electron energy was 6 MeV, applied pulse duration 4 μs, frequency 20 Hz, dosing rate 6 kGy/min and a gamma radiation dosing rate of 3,4 kGy/h.

Results and Discussion

The obtained nanofibrous scaffolds based on hyaluronic acid that were obtained by electrospinning process with the selected process conditions, were subjected to microscopic structure analysis. The behaviour of the porous structure (the spaces between the fibres) provides access for physiological fluids and suitable cells to active substances. Despite the rapid dissolution process of the substrates in the body, it creates the possibility of proper distribution of the bioactive substance in the vicinity of diseased tissue or cavities. FIG. 1 presents sample images of hyaluronic acid substrates before and after the sterilization process.



A)



B)

FIG. 1. SEM images of hyaluronic acid-based scaffolds: A) before the sterilization process; B) after the sterilization process.

Conclusions

The research revealed that under the influence of the radiation dose of 25 kGy used during the sterilization process, no changes in the structure of microscopic substrates were observed. However, further studies to determine changes in the chemical structure of compounds used in necessary.

Acknowledgments

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THE EFFECT OF BIOACTIVE GLASS PARTICLE SIZE ON PROPERTIES OF POLY(ε-CAPROLACTONE) BASED MEMBRANES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 66]

Introduction

The guide bone regeneration (GBR) involves application of barrier membranes to prevent soft-tissue ingrowth into the defect and to maintain a suitable space for bone regeneration process [1]. A membrane should actively promote bone formation and membrane-tissue integration that stabilizes the healing wound process [2]. It has been shown that the incorporation of bioactive glass (BG) particles into polymeric membranes can significantly enhance osteoblast proliferation and differentiation, indicating favourable osteoconductivity and/or osteoinductivity for GBR applications [1] and also can induce direct bone-material bonding [3].

The current study aimed to evaluate the effect of BG particle size (<3 μm, <45 μm) on properties of poly(ε-caprolactone) (PCL) membranes obtained by two methods, namely thermal-induced phase separation (TISP) and liquid-induced phase separation (LIPS).

Materials and Methods

BG particles with the composition of $40SiO_2$–$54CaO$–$6P_2O_5$ (mol%) were synthesized with the use of sol-gel method. PCL/BG membranes were prepared by TIPS and LIPS methods using 5%w/v PCL solutions in 1,4-Dioxane (DIOX) and N,N-Dimethylformamide (DMF), respectively. The assumed volume fraction of BG particles in composites was 21 vol.%. Both surfaces of films were evaluated in terms of surface properties: morphology (SEM), topography (confocal microscopy), wettability (contact angle goniometer). Also degree of crystallinity and melting point (DSC) of PCL were examined. *In vitro* bioactivity was assessed by incubation of materials in simulated body fluid (SBF) for 3, 7 and 14 days. The samples and incubation media were analysed with SEM/EDX, FTIR and ICP-MS methods, respectively.

Results and Discussion

The results showed that the use of BG particles of various sizes (<3 μm, <45 μm) affects surface properties such as morphology, topography and wettability; as well as *in vitro* bioactivity of the PCL/BG membranes obtained by two different methods.

In the case of materials obtained with LIPS method, the presence of BG particles in PCL matrix resulted in larger pore size in comparison with pure polymer membrane. However, material containing larger-sized particles (<45 μm) showed bigger pores than membrane with BG particles of <3 μm size. In turn, porosity and pore size of membranes, produced with TIPS method, decreased with the addition of glass particles into PCL matrix. Moreover, larger-sized particles (<45 μm) caused greater reduction in porosity and pore size.

The static water contact angle and water adsorption of membranes obtained with the use TIPS method were not affected by the presence of glass particles. On the contrary, the addition of BG particles of both sizes into membrane produced by LIPS method improved wettability and water adsorption. However, in the case of material containing glass particles of <3 μm size, more noticeable improvement was seen.

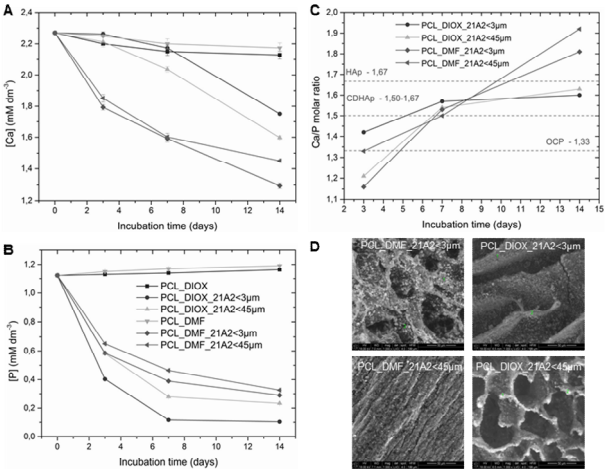

FIG. 1. Variations of the Ca (A) and P (B) concentrations in SBF during material soaking; Ca/P molar ratio (C) of the formed layer after material incubation in SBF; SEM images (D) of the materials after 14-day incubation in SBF.

All of the polymer membranes showed no significant changes in surface morphology and chemical composition after soaking in SBF. In turn, the surfaces of PCL/BG materials were fully covered with the thick layers rich in calcium and phosphorus (FIG. 1D). All of the layers exhibited spherical cauliflower-like morphology, typical of carbonated hydroxyapatite (HCA), as was additionally confirmed with FTIR spectroscopy. The results of Ca and P concentration in SBF during material soaking (FIG. 1A-1B) and also Ca/P molar ratio of the formed layers (FIG. 1C) indicated that kinetics of *in vitro* precipitation of HCA depends on both membrane preparation method and BG particle size. It was shown that after 7 days of incubation, for all composite materials, Ca/P molar ratio is characteristic of calcium-deficient HAp (CDHAp). However, after 14-day incubation, materials obtained by TIPS still show Ca/P ratio characteristic of CDHAp, while membranes produced with the use LIPS exhibit Ca/P ratio above value typical for Hap, which can indicate the formation of B-type carbonate-substituted hydroxyapatite (PO_4^{3-} substituting a tetrahedral group with CO_3^{2-}).

Conclusions

The results indicate the possibility of using various membrane preparation methods and also BG particles of different sizes to obtain materials with various, but controlled surface properties, as well as *in vitro* bioactivity.

Acknowledgments

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THE GROWTH OF SAOS-2 CELLS ON DLC LAYERS DOPED WITH TITANIUM

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[ENGINEERING OF BIOMATERIALS 138 (2016) 67]

Introduction

Diamond-like carbon (DLC) coatings have been widely studied due to their potential usage as a coating of implants. DLC layers can be doped with various chemical elements, e.g. chromium, titanium, cobalt, and silver. Titanium(Ti)-doped or chromium(Cr)-doped DLC layers were observed to improve properties of the material internal stress, to reduce peeling from the material surface and risk of cracking [1]. Saos-2 cells are a human osteoblast-like cell line that has a potential to undergo osteogenic differentiation [2]. In this work, we evaluated the adhesion, growth, and osteogenic differentiation of Saos-2 cells on DLC layers enriched with different Ti content.

Materials and Methods

The Ti-DLC layers were prepared of a different Ti content on glass as follows: 0.0, 0.7, 3.3, 5.2, 10.0, 24.5 at% Ti. The glass coverslip (Gl) and tissue culture polystyrene (PS) were used as control materials. Prior cell seeding, the samples were sterilised in 70% ethanol for 2 hours. The Saos-2 cells were seeded in a density of 20,000 cells/cm^2 and cultured in McCoy's 5A medium with 15% foetal bovine serum for 7 days. On day 1, 3, and 7, the cells were fixed by 70% frozen ethanol and stained with Texas Red C_2-maleimide (day 1), stained for vinculin using primary mouse anti-vinculin antibody and secondary anti-mouse antibody, and for F-actin using phalloidin conjugated with TRIC (day 3), and for osteopontin (day 7) using primary mouse anti-osteopontin antibody and secondary anti-mouse antibody. The cell nuclei were counterstained with Hoechst 33258. The cell spreading area was measured from microphotographs of 289–399 cells stained with Texas Red C_2-maleimide (day 1) using Atlas Tescan software. The cell number was counted from microphotographs of immunofluorescence staining taken on day 1, 3, and 7. All microphotographs were taken under epifluorescence microscope Olympus IX 71 microscope, objective ×20 and ×10. The data is expressed as mean + SEM. ANOVA, Dunn´s method. The statistical significance ($p<0.05$) is specified above the columns (compared to every sample on the same day of the culture).

Results and Discussion

The number of initially adhered cells on day 1 was from 7,927 cells/cm^2 on DLC without Ti to 12,871 cells/cm^2 on glass sample. The cell number on day 3 ranged from 20,465 cells/cm^2 on DLC without Ti to 45,832 cells/cm^2 on PS sample. The cell number increased during the time period and on day 7, the cell number reached values from 98,887 cells/cm^2 on DLC with 5.2 at% of Ti to 176,815 cells/cm^2 on DLC without Ti. On day 1, the highest cell spreading area (FIG. 1), was observed on DLC with 5.2 at% of Ti and the lowest was on glass. On all DLC-based and PS samples, the cells were polygonal-shaped; however, on the control glass sample, the cells were rather round-shaped. On day 3, staining for vinculin showed a more apparent assembly of vinculin-containing focal adhesion plaques on samples with a higher Ti content (FIG. 2 A). On day 7, as the cells on all samples reached confluence, we found that the osteopontin, a marker of osteogenic differentiation, is present in Saos-2 cells on all Ti-DLC (FIG. 2 B) and control samples.

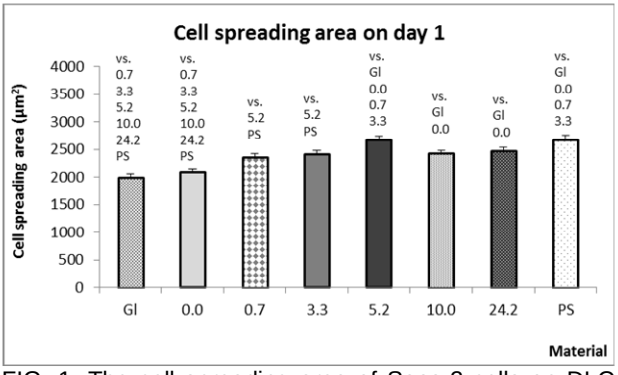

FIG. 1. The cell spreading area of Saos-2 cells on DLC doped with 0.0, 0.7, 3.3, 5.2, 10.0, 24.5 at% of Ti, on glass (Gl), and on polystyrene (PS) on day 1. The data is expressed as mean + SEM. ANOVA, Dunn´s method. The statistical significance ($p<0.05$) is specified above the columns (compared to every sample on the same day of the culture).

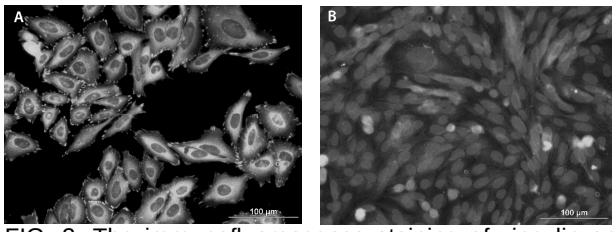

FIG. 2. The immunofluorescence staining of vinculin on day 3 (A) and of osteopontin on day 7 (B) in Saos-2 cells on DLC with 5.2 at% of Ti. The cell nuclei are counterstained with Hoechst 33258, Olympus IX 71 microscope, objective ×20.

Conclusions

We proved that Saos-2 cells adhered and proliferated during the followed time period of 7 days on Ti-DLC samples as well as on control samples. According to the cell spreading area and the presence of more apparent assembly of vinculin-containing focal adhesion plaques, the samples with higher Ti content seem to be more suitable for quick adhesion of the Saos-2 cells. The osteogenic differentiation was confirmed by the presence of osteopontin.

Acknowledgments

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PLASMA OXIDIZED Ti6Al4V AND Ti6Al7Nb ALLOYS FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 68]

Introduction

Titanium and its alloys besides many advantageous mechanical properties and high corrosion resistance is characterized by high coefficient of friction (in combination with almost all materials), low resistance against wear and a tendency to galling. This substantially limits the use of titanium and its alloys form components operating in conditions of friction. Alloying additions and heat treatment also do not improve their tribological properties.

In order to fully exploit the potential of titanium and its alloys the use of thermo-chemical treatment has been applied. Surface modification of titanium and its alloys is carried out mainly through incorporation into the surface layer such element as: N, O, C, B, Si, which form the corresponding compounds of solid solutions [1-5]. In this work are presented the results of the influence of the plasma oxidizing of titanium alloys on the evolution of its structure, mechanical and corrosion properties.

Materials and Methods

Two titanium medical grades: Ti6Al4V and Ti6Al7Nb were subjected to glow discharge oxidation. After the modification both substrate materials were characterized in terms of structure, thickness of the oxygen diffusion zone, distribution of hardness and surface geometrical structure. Samples were subsequently tested in the tribological tests using the ball on disc method with zirconium oxide as a counter sample. After the tests the coefficients of friction were determined and wear rates calculated for both samples and ZrO_2 balls. The corrosion measurements were made with use of electrochemical methods. The corrosion potential E_{cor} was measured in an open circuit (OCP) while recording the potential of the sample relative to the reference electrode for 1800 s. The value of polarization resistance, R_p, was determined according to Stern–Geary method in a scanning range of ± 20 mV vs. E_{cor} potential at the rate of 0.3 mV/s. Potentiodynamic characteristics were measured in a wide range of anodic polarization starting at potential E_{cor} – 0.2V V to 4V with the scan rate of 1 mV/s.

Results and Discussion

The conducted processes of diffusion strengthening of titanium alloys by interstitial oxygen atoms positively influenced the investigated properties. We managed to increase twice the hardness of the surface of the tested alloys and the thickness of the diffusion zone was estimated to be approx. 85 µm. These parameters resulted in a reduction of the wear rate determined in ball on disc tests. The registered value of the wear rate of the Ti6Al4V alloy decreased by one order of magnitude, whereas for the Ti6Al7Nb alloy it was 7 times lower. The lower values of wear rate were achieved despite the fact, that the friction coefficients, compared to the unmodified alloys, have increased from 0.45 to 0.7. The plasma oxidation of Ti alloys favorably affected their corrosion resistance. The value of the corrosion potential significantly increased (approx. 0.36 V). At the same time the polarization resistance increased three times for the Ti6Al4V alloy and 10 times for the Ti6Al7Nb alloy, which demonstrates the better corrosion resistance of the modified samples. The results of the potentiodynamic studies also confirmed a high resistance of the modified alloys against the pitting corrosion. As a drawback of the process of oxidation an increase in surface roughness can be pointed out. It results in the need of additional polishing treatment restoring the original surface smoothness and removing a thin surface layer of a porous oxide.

Conclusions

In summary, it can be stated that the plasma oxidation of titanium alloys favorably influenced the tribological and corrosion properties of both Ti6Al4V and Ti6Al7Nb alloys.

Acknowledgments

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INFLUENCE OF CHEMICAL COMPOSITIONS ON THE STRUCTURE AND THE BIOACTIVE PROPERTIES OF SiO_2-CaO AND SiO_2-CaO-P_2O_5 BIOACTIVE GLASSES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 69]

Introduction

Bioactive glasses are the materials known for their ability to create a bond with a bone tissue through the layer of carbonated apatite. They are also known for their biocompatibility, osteoconductive and even in some cases osteinductive properties [1]. The bioactivity of glasses is very complexed issue and depends on many different factors such as chemical composition, texture or surface properties. Aim of that study was to evaluate the influence of materials chemical composition on the structure and bioactivity, described as an ability of creating a layer of apatite on materials surface in contact with simulated body fluid *in vitro.* There were two main compositional variables considered: first of all value of CaO/SiO_2 ratio and also presence of phosphorus in materials composition.

Materials and Methods

Bioactive glasses from the binary SiO_2 - CaO and ternary SiO_2 - CaO - P_2O_5 systems were obtained by the sol-gel process. CaO/SiO_2 molar ratio in obtained materials varied between 0.2 to 1.5. Dried gels were heat treated in the temperatures 600ºC (binary glasses) and 700ºC (ternary glasses) for 20h. All of the obtained powders were subjected to the structural analyses. XRD analysis was performed in order to evaluate degree of crystallinity of glassed. Moreover, FTIR analysis has been made due to characterization of the structure of obtained glasses.

The local structure of silicon and phosphorus (ternary glass system) in obtained materials were examined with magic angle spinning nuclear resonance (MAS-NMR). Moreover, the optical basicity parameter was calculated according to the NMR data. Bioactivity of powders was assessed during the incubation in simulated body fluid (SBF) test. After 7 days of incubation powders were analyzed with XRD and FTIR to qualify changes in materials structure occurred in the contact with incubation fluid.

In order to quantify the concentrations of calcium, phosphorus and silica that dissolve from glass powders during the incubation, ICP-OES spectroscopy was performed. Glass powders of the particles size < 45um were closed into biodegradable, polymer (PLGA) matrix. The weight ratio of glass particles to the polymer was 1:1. The experiment was conducted in SBF and particular ions concentrations were measured after 3, 7 and 14 days.

What is more, measurements of ion dissolution form samples pre-covered with apatite were conducted. At first, samples were incubated for 3 or 7 days in SBF solution and then were immersed in α-MEM cell culture medium supplemented with Fetal Bovine Serum (FBS). Medium was collected and tested after 24h, 48h, 4 and 7 days respectively.

Results and Discussion

XRD analysis revealed the beginning of crystallization in majority of glasses. The intensity of that process increased with the increase in CaO/SiO_2 ratio. Crystallized phases were calcium silicates (binary and ternary glasses) and hydroxyapatite (ternary glasses). FTIR spectra showed changes in the materials structure depending on the CaO/SiO_2 ratio. It has been proven that with the increasing CaO/SiO_2 ratio the number of bridging oxygens significantly decrease. That can suggest that higher content of modifier, such as calcium, in the glass structure causes a decrease in glass network polymerization.

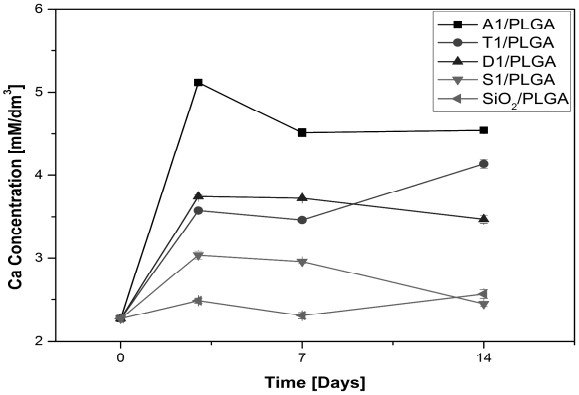

FIG. 1. Changes in calcium concentrations in SBF solution after 3, 7 and 14 days of samples incubation.

The ^{29}Si MAS-NMR spectra revealed that silicon was present in Q^2, Q^3 and Q^4 structural units. Moreover, ^{31}P MAS-NMR indicated that phosphorus exist mainly as a monophosphate complex. It has been also shown that the presence of phosphorus in the structure induct the process of silica network repolymerization. Optical basicity was increasing with a rise of CaO/SiO_2 ratio [2].

Changes in powders structure after incubation in SBF have been indicated. XRD analysis revealed carbonate apatite and calcite crystallization. These results were confirmed by FTIR analysis. What is more, main phase crystallized in the binary system glasses was calcite otherwise, main phase appeared in ternary glasses was carbonated apatite.

ICP-OES analysis revealed that particular ions concentrations in SBF had been changing in time (FIG. 1). The character of changes depended strongly on the glass composition. Analysis of concentrations of ions released to immersion fluid from materials pre-covered by HCA layer indicated different dynamics of release in comparison with not pre-covered materials.

Conclusions

Our study has confirmed that chemical composition of bioglasses affects the glass structure as well as the process of bioactivity. We have proven that it is possible to modulate the chemical properties of this kind of composites depending on desires application.

Acknowledgments

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

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SELENIUM CONTAINING HYDROXYAPATITE GRANULES AS DRUG CARRIERS FOR RISEDRONATE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 70]

Introduction

Substituted hydroxyapatites have many applications from optoelectronics to biomaterials for regenerative medicine and dentistry [1]. It is known that partial ionic substitutions may change physicochemical, biological or mechanical properties of apatites. Selenium is an essential microelement playing a significant role in many metabolic processes [2]. Its anticancerogenic activity and beneficial impact on the inflammatory response of osteoblasts in the metastasis of certain bone tumours have been reported. Risedronate sodium is a drug from the group of bisphosphonates. It slows bone loss and is commonly used in osteoporosis and Paget's disease treatment [3]. It inhibits bone metastasis and can be used in certain bone tumours treatment.
In this work, we prepared selenite (SeO_3^{2-}) enriched hydroxyapatite to produce porous granules for risedronate release.

Materials and Methods

SeO_3^{2-}-containing hydroxyapatite (Se-HA) was synthesized by the standard wet method [4]. The obtained precipitates were aged, centrifuged, washed and dried at 130°C. The powders were physicochemically studied by using powder X-ray diffractometry (PXRD), infrared spectroscopy (FT-IR), atomic absorption spectrometry (AAS). The powder was then granulated with the use of 4% alginate sodium aqueous solution and hydrogen ammonium carbonate (0-5 wt. %). The hydroxyapatite/alginate ratios were optimized. During the granules formation, a solution of risedronate sodium was added into the alginate solution. After that, the dense suspension was squeezed out into small spherical drops by a syringe needle and added to 1.5% solution of $CaCl_2$. These alginate/apatite granules were washed with water and dried in air at 40°C for 24h.
Solid-state MAS NMR (^{13}C, ^{31}P, ^{1}H) was used for structural analysis of Se-HA and porous composite granules.

Results and Discussion

PXRD diffractograms have shown that the initial powders are nanocrystalline hydroxyapatites without other crystalline phases. The obtained crystallites are plate-like shaped. Selenium content was calculated as 7.5 wt%. Selenite ions are located in the crystalline core and in the hydrated surface layer. The porous beads size and the pore microstructure characteristics were analysed with scanning electron microscopy (SEM; FIG. 1). ^{31}P MAS NMR was used for specific surface area estimation ($SSA_{Se\text{-}HA}$= 150 m^2/g). In ^{31}P CP MAS spectra the characteristic signals from hydroxyapatite (at ca. 3.2 ppm) and from risedronate (a broad signal at ca. 19 ppm) are visible. The risedronate present in the granules reacted with calcium cations. In ^{13}C CP MAS NMR spectra we can observe the signals from alginates and risedronate.

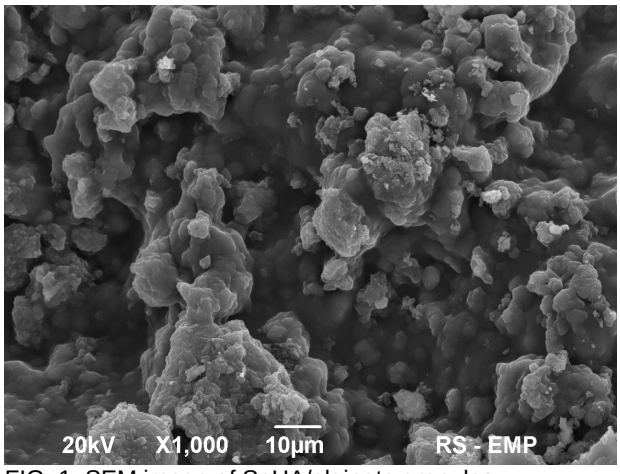

FIG. 1. SEM image of SeHA/alginate granules.

Conclusions

Nanocrystalline hydroxyapatite doped with selenite ions was successfully prepared and used for porous granules production. The composite beads contained risedronate adsorbed on the apatitic crystals in amount detectable in NMR experiments. Future studies will focus on evaluation of drug release and biological tests.

Acknowledgments

This work was supported by the research program (Project NCN-2011/03/D/ST5/05793) of the National Center of Science, Poland.

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INFLUENCE OF THERMO-CHEMICAL TREATMENT METHODS ON PITTING CORROSION RESISTANCE OF Ti6Al4V ALLOY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 71]

Introduction

Thermo-chemical treatments such as oxidation, carburization or nitriding are used in order to improve the mechanical properties of materials, especially the tribological superficial one. This parameter is particularly important for metallic biomaterials, which are used for implants production. Biomaterials working in a pair, such as endoprosthesis in a hip-joint, are particularly exposed to wear friction, so they should have a very high tribological strength. Additionally, the body fluids consist very harsh environment for biomaterials and can reduce the corrosion resistance of implants. That leads to a pitting corrosion. When it develops implant must be removed from the body, because the products of corrosion can damage the surrounding tissues. An important aspect for this is therefore to keep balance between the thermo-chemical treatment and corrosion resistance of the material. The results present in this work show the influence of thermo-chemical treatments on the corrosion potential and pitting corrosion of the Ti6Al4V alloy.

Materials and Methods

Samples made of Ti6Al4V alloy were superficially diffusive strengthen through the following thermo-chemical treatments: oxidation, carburization and nitriding. The processes were carried out in a wide temperature range from 650ºC do 950ºC. Next the samples were investigated towards corrosion properties. Corrosion tests were done in deoxygenated PBS solution (pH 7.4) using potentiostat/galvanostat AUTOLAB 302N (Metrohm Autolab B.V.) controlled by NOVA 1.11 software. Saturated calomel electrode was used as reference, while a platinum wire served as the auxiliary electrode. The corrosion potential was measured in open circuit potential for 1800s. The resistance to pitting corrosion was evaluated by anodic polarization up to potential value of 4V (the scan rate was 1.0 mV/s). After polarization tests the samples were photographed and their surfaces were examined using metallographic microscope.

Results and Discussion

The corrosion resistance of materials strongly depends on their surface chemistry which is correlated with the corrosion potential (E_{cor}) value. Therefore some initial information about corrosion properties of investigated materials may be extracted from the comparison of E_{cor} values determined before and after applied surface treatments. In this study all applied thermo-chemical treatments resulted in higher value of corrosion potential (E_{cor}). The highest E_{cor} value was determined for Ti6Al4V alloy sample carburization at temperature of 850ºC.

The effect of thermo-chemical surface treatments on pitting corrosion resistance of titanium alloy was estimated on the basis of potentiodynamic characteristics gathered in a wide range of anodic polarization. Such characteristics representative for each type of investigated samples are shown in FIG. 1. These characteristics allowed to determine the pitting potential (E_{pit}), which was taken as the potential value, where an abrupt increase in the anodic current density was observed. From FIG. 1 it is evident that titanium alloy without surface treatment, as well as alloy samples oxidized and gas nitrided at lower temperature are pitting resistant. In the case of these samples no pitting corrosion could be detected. While for the other samples the pitting occurred, and E_{pit} potential was detected in the range of 2.0-2.5V. The changes in surface morphology caused by anodic polarization were different depending on type of sample. The photographs of the investigated samples taken after anodic polarization are presented in FIG. 2.

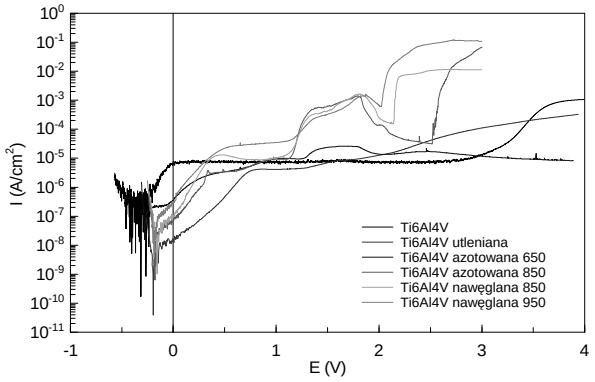

FIG. 1. The anodic polarization curves for thermo-chemical treated Ti6Al4V alloy samples.

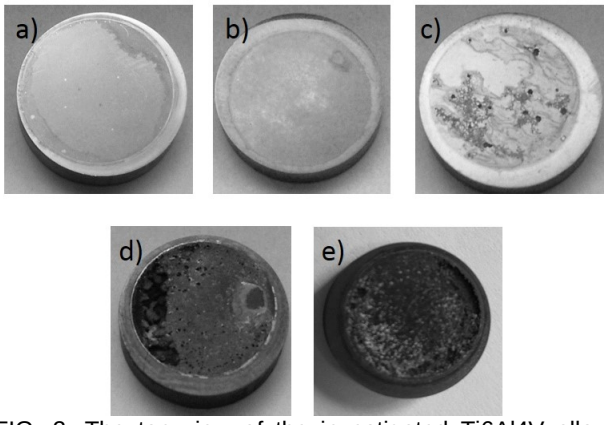

FIG. 2. The top view of the investigated Ti6Al4V alloy samples after anodic polarization a) oxidized, b) gas nitrided 650ºC, c) gas nitrided 850ºC, d) gas carbourized 850ºC, e) gas carbourized 950ºC.

Conclusions

Taking into consideration the obtained results it can be pointed that all the applied surface treatment processes alter the corrosion properties of Ti6Al4V alloy. Gas nitriding at higher temperature, as well as gas carburizing regardless on process temperature increase the pitting sensitivity of titanium alloy. Nevertheless the pitting corrosion resistance of such samples is still sufficient for biomedical application since pitting potentials are very high (above 2V).

Acknowledgments

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NiTi SHAPE MEMORY CLAMPS FOR BONE FRACTURE TREATMENT IN RABBIT: PRELIMINARY REPORT

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[ENGINEERING OF BIOMATERIALS 138 (2016) 72]

Introduction

Most of the fractures of the long bones in rabbits can be repaired using an intramedullary pin and orthopedic wires. However placing the cerclage wire around the fracture site can damage the last vestiges of blood supply from the bone fragments.
As an alternative to that clamps made of the NiTi shape memory wire can be applied. The NiTi alloy is well known from its medical applications [1-3]. It is due to its shape memory effects, high biocompatibility, acceptable resistance to corrosion, especially, when it is applied for a short-term staying in biological environment [4-5].
In presented work, as a support for classical technique for bone fracture fixation, the clamps made of NiTi shape memory were applied.

Materials and Methods

The clamps, with their shape and size, shown in FIG. 1, were prepared from the NiTi wire provided by BHH Mikrohuta (Dąbrowa Górnicza, Poland). The wire was 1.2 mm in diameter.

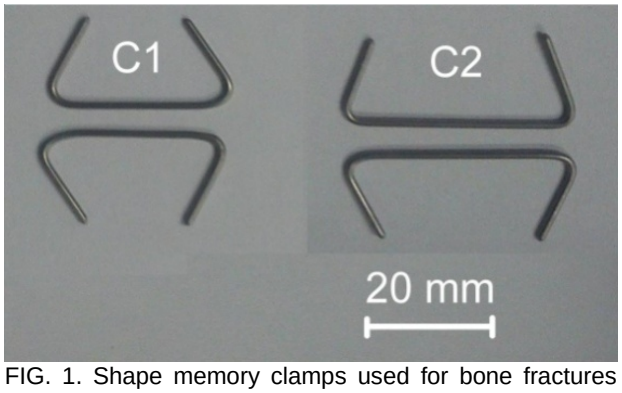

FIG. 1. Shape memory clamps used for bone fractures fixing.

The experiments were carried out on 6-months old rabbit. After the tibial osteotomy, intramedullary fixation was used to stabilize the fracture, the ends of the fracture were secured by one of two kinds of clamps marked as C1 and C2. In order to receive proper stabilization of the bone fragments, they were mounted in different directions. The length, of the working arms of the clamps, was adjusted to a bone diameter.
Thermal range of the reversible martensitic transformation, occurring in NiTi wire, was studies with use of the differential scanning calorymetry (DSC).

Results and Discussion

In order to ensure that clamps are able to reveal shape memory phenomena, the presence and course of the martensitic transformation was studied. Thermograms measured at thermal range between -120°C and 50°C (FIG. 2). It can be found that one maximum and one minimum is present on cooling and heating DSC curve, respectively. Martensitic transformation starts at -35°C (M_s) and finishes at -80°C (M_f), whereas reversible transformation starts at -28°C (A_s) and finishes at -9°C (A_f). The ability to received force from the transformation is determined by enthalpy of transformation. In all clamps its value was about 13 J/g. Obtained parameters of the reversible transformation enabled application of the one-way shape memory effect.

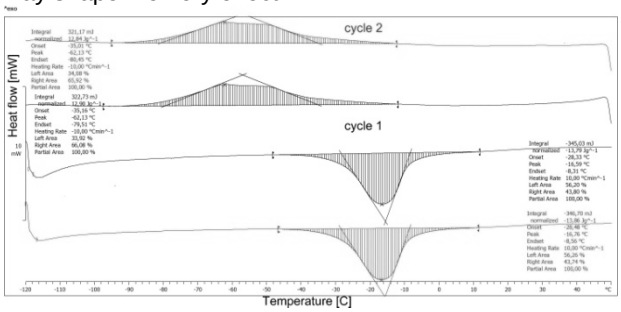

FIG. 2. DSC cooling/heating curves measured for wire used for clamps production.

All these results were used for set up the medical operation. First, clamps were sterilized and cooled down to temperature of liquid nitrogen (below M_s). At that temperature, the arms were bent to 90° position. Then, clamps were fixed in previously drilled holes in both fractures of the broken bone. In result of using heat of the rabbit's body, one-way shape memory effect caused fixation of the bone fragments (FIG. 3).

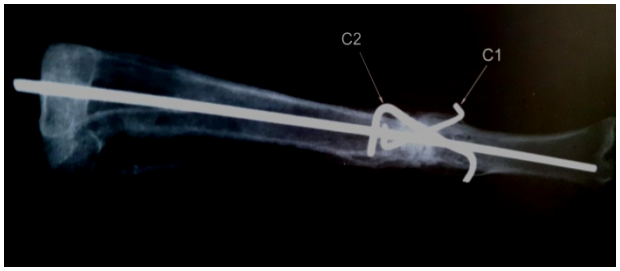

FIG. 3. Radiograph showing fixed bone fracture with use of clamps C1 and C2.

All fractures were radiologically healed at 6 weeks, however clamps were intentionally left for next 6 weeks. After that, the tissue surrounding channel, after clamp removal, was examined. Results proved that no anatomical changes were found.

Conclusions

The NiTi clamps appeared to be alternative supportive way for bone osteosynthesis applied to rabbit. Moreover, bone fractures after 6 weeks were correctly healed.

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EVALUATION OF CELLULOSE/HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING: STUDIES *IN VITRO* AND *IN VIVO*

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[ENGINEERING OF BIOMATERIALS 138 (2016) 73]

Introduction

Nowadays, development of three-dimensional (3D) scaffolds for bone regeneration is one of current challenges in the tissue engineering. A variety of materials are proposed for the fabrication of 3D scaffolds. The main requirements for such scaffolds are as follow: (i) the structure similar to that of a natural bone; (ii) interconnected pores suitable for fast nutrition and metabolites diffusion; (iii) biocompatibility; (iv) osteoconductive and osteoinductive properties [1].

The aim of this work was to evaluate novel cellulose-based scaffolds with immobilized microhydroxyapatite (cellulose/μHA) and nanohydroxyapatite (cellulose/nHA) for the bone tissue engineering. Previously it was shown that the morphology of the scaffolds, i.e. porosity, pore size, framework thickness, corresponds to that of natural bone [2]. In this work results of *in vitro* and *in vivo* studies are presented.

Materials and Methods

Biocompatibility and potential toxicity of the cellulose/HA scaffolds were tested by determination of cell viability, cell membrane integrity and the response to insulin. Hepatocytes and the extensor digitorum longus muscle tissue isolated from rats were used for the studies. Membrane integrity was tested by evaluating of the lactate dehydrogenase (LDH) and aldolase release from the cells after their incubation with the scaffolds. Metabolic effects of cellulose/HA composites were studied by evaluating liver and muscle cells sensitivity to insulin after 90 min incubation with the composite samples. To analyze the insulin-induced glucose uptake by the cells the 2-D-[^{3}H] glucose was used.

In vivo studies were performed using the mouse as well as the rabbit model. The scaffolds were implanted subcutaneously in the back of mice and harvested after 2 weeks, 1 and 3 months of the implantation for the histological examination. Using rabbit model the scaffolds were implanted into a calvaria bone and harvested after 2, 4 and 12 weeks after the implantation. Then X-ray spectroscopy, microcomputed tomography and histology were used for the examination of the samples.

Results and Discussion

In vitro studies with hepatocytes and the extensor digitorum longus muscle tissue have confirmed that the cellulose/μHA scaffold is not cytotoxic and can be used in contact with biological systems. However, the cellulose scaffolds containing nanoparticles have decreased liver cell viability and increased the release of lactate dehydrogenase and aldolase from hepatocytes and extensor digitorum longus muscle myocytes, respectively (FIG. 1).

Moreover, the cellulose/nHA scaffold significantly reduced the insulin stimulated glycogen synthesis in the liver cells and glucose uptake by myocytes.

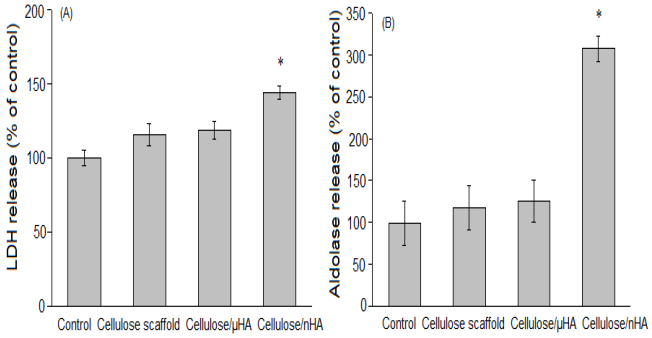

FIG. 1. Effect of the scaffolds on LDH (A) and aldolase (B) release in the incubation medium of cells.

In vivo studies with mice did not show significant differences between cellulose/μHA and cellulose/nHA. In both cases no wound complications were observed. After 2 weeks of implantation, the histological analysis showed that there was the inflammatory response of the surrounding tissue. The scaffold was surrounded with a fibrous and collagenous tissue capsule. The histological analysis of specimens harvested after 1 and 3 months revealed the biocompatibility of the scaffolds. The connective tissue proliferated within the scaffolds, angiogenesis was also expressed.

In vivo studies with rabbits revealed osteoconductive properties of the scaffolds. The scaffolds induced a fast new bone formation. After 12 weeks of implantation appropx. 20 % of a newly formed bone within the defect was observed (FIG. 2).

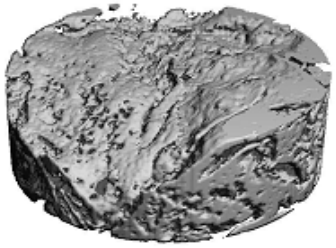

FIG. 2. 3D image of cellulose//μHA scaffold after 12 weeks of implantation in the rabbit calvaria.

The results were similar to those obtained with commercial allogenic and xenogenic bone blocks implanted for comparison.

Conclusions

The *in vitro* and *in vivo* studies showed that the cellulose//μHA scaffold is osteoconductive and non-cytotoxic. Thus, it has a high potential to be used as an implantable material in bone defects. However, cellulose scaffolds with nanosized HA particles have demonstrated slight cytotoxicity in the studies *in vitro*.

Acknowledgments

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IN VITRO EVALUATION OF SELECTED PROPERTIES OF NEW FLOW-TYPE DENTAL COMPOSITE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 74]

Introduction

Dental composites used as restorations are required to have long-term durability in the oral cavity. In some cases the interaction of these materials with oral fluids may involve dissolution or degradation of materials while in others the interaction may cause an uptake of fluids into the structure of the material which may affect the mechanical properties of the materials [1-3]. The formation of deposits on the dental restorations was not quite well recognized yet.
In this study, the properties of new experimental flow-type dental composite and its behavior in artificial and natural saliva environment in comparison to commercial materials was investigated.

Materials and Methods

Materials used in this study were:

- experimental composite (36% wt. of resins (Bis-GMA, EBADMA); and 64% wt. of fillers (Ba-Al-Si glass and nanosilica), marked as "*Exp*".
- Flow Art (Arkona) (38% wt. of Bis-GMA, UDMA, TEGDMA i Bis-EMA) and 62% wt. of fillers: Ba-Al-Si glass and nanosilica), marked as "*FA"*,
- Charisma Opal Flow (Heraeus) (UDMA and EBADMA resins and 65% wt. of fillers: Ba-Al-F silicate glass, YbF_3 and SiO_2), marked as "*Ch*".

All materials had shade of A1.
Specimens for testing were made according to ISO 4049 as bars of dimensions 2x2x25 mm. Materials were cured using halogen lamp (Cromalux 75, Mega-Physik) via Mylar strips. Microhardness measurements (Vickers) were taken on cured surface using tester FM-800 (Future-Tech Corp.) with the load of 0.49N. Flexural test was made using Zwick Z2.5 universal testing machine, conditions of the test were according to ISO 4049.
After curing all specimens were placed in distilled water in the incubator (37^{o}) for 24 hours. Next they underwent reference mechanical tests. The rest of the specimens were placed in eppendorf tubes (2 cm^3) filled with natural saliva (marked "*n"*) (collected by a volunteer) and artificial saliva (marked "*a"*) (ISO 10271). Next tests were made after 28 days of incubation (and also 7 days in the case of microHV). Results were statistically analyzed using Statistica software (StatSoft Inc.).
Surface of the specimens was also tested by FTIR (Hyperion 3000 with Vertex 70, Bruker) for formation of deposits.

Results and Discussion

In the case of strength (FIG. 1), no statistically significant differences for *Ch* and *FA* materials were observed. For *Exp* material, the statistically significant diminishing in strength was observed in both media; however, it still exceeds required value described in ISO 4049. Due to the relatively short period of incubation, Authors did not notice significant changes in flexural strength. These tests are continued at present.

Microhardness (FIG. 2) showed a significant initial increase in each case due to a dark phase polymerization [5]. In the long term of incubation, the microhardness significantly diminished due to hydrolytic degradation of resin matrix [2].

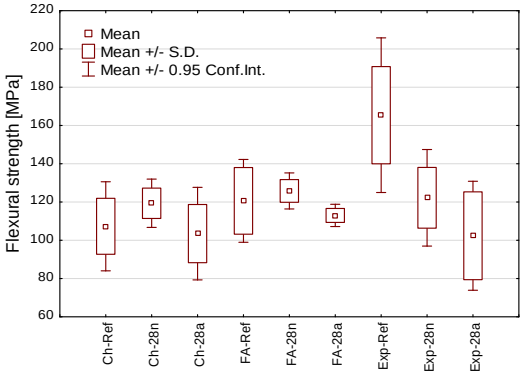

FIG. 1. Flexural strength of tested materials.

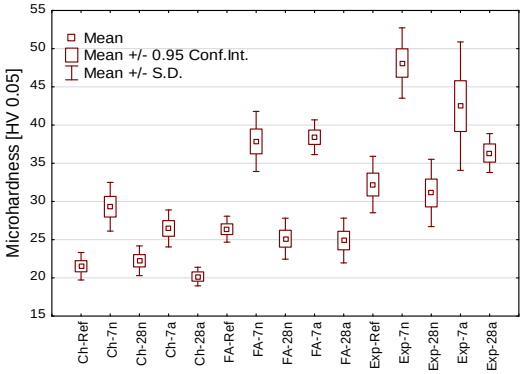

FIG. 2. Microhardness of tested materials.

FTIR analysis (FIG. 3) showed the formation of carbonated calcium phosphates deposit only in the case of artificial saliva environment. Natural saliva, probably due to inter-individual variability of composition, pH and also the presence of enzymes (affecting pH), did not produced any significant deposit.

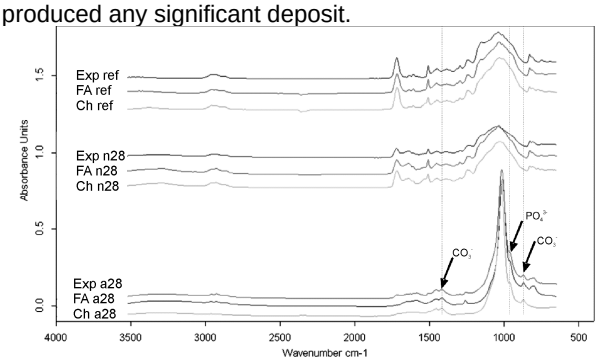

FIG. 3. FTIR analysis of deposits.

Conclusions

Experimental material showed a slight diminishing in strength due to lower resistance for hydrolytic degradation. Microhardness of *Exp* material was similar to *FA* and higher than *Ch* materials. Natural saliva did not cause formation of any deposits during the test.

Acknowledgments

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POLYMERIZATION SHRINKAGE OF NEW FLOW-TYPE DENTAL COMPOSITE USING MICRO-CT

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[ENGINEERING OF BIOMATERIALS 138 (2016) 75]

Introduction

Polymerization shrinkage of the resin-based dental composites constitutes a risk of the failure of the interfacial bonds as a result of shrinkage stresses. It may result in marginal leakage, premature failure of the restoration, and even micro-cracking of the tooth [1,2]. Therefore, the research for develop a low shrinkage material has been a goal in the manufacture of dental composites.
The color restorative materials are very interesting and market demand for these products was increased recently. They are used especially in milk tooth as fissure sealing, for marking root canal openings or as decoration (tooth tattoo) [3].
In this study, the research of polymerization shrinkage of flow-type dental composites was conducted.

Materials and Methods

Materials used in this study were:

- Flow-Art (Arkona) (38% wt. of Bis-GMA, UDMA, TEGDMA and Bis-EMA) and 62% wt. of fillers (Ba-Al-Si glass and nanosilica), marked as "*FA shade"* (shade = A1, A2 or A3),
- Flow-Color (Arkona) (the same composition as above + pigments), marked as "*FC colour" (colour = orange, yellow, blue, green, violet and pink, in ascending order of curing' depth)*

Volumetric shrinkage was measured using micro-CT scanner Skyscan 1174 (Bruker microCT) with accuracy of 6.5 µm. A drop of composite material was shaped into a semi-sphere on the tip of the Teflon pin of diameter 3 mm (FIG. 1). Volume of material used was about 3 mm^3. Scanning was started after 3 min. to allow material spreading on the tip surface and get a spherical shape. Samples were scanned in angular range of 0-180° with step of 1°. Then composite was cured (for the time specified by manufacturer) using halogen lamp (Cromalux 75, Mega-Physik). The tip of the gun was positioned 2 mm above the sample. One minute after curing the next scan was started to obtain volumetric data of cured material [3]. After reconstruction the volume of specimen was obtained before and after curing. The volumetric shrinkage was calculated as the ratio of difference between uncured and cured material volume to uncured composite volume. Each composite was measured 10 times and results were statistically analyzed using Statistica software (StatSoft Inc.).

Results and Discussion

Flow-Art composites had a volumetric shrinkage of about 3.2% (FIG. 2) and there was no significant differences observed in relation to shade.
Coloured composites (*FC*) had the same basic components (mix of resins and fillers) and the main difference was the pigment addition, which resulted in different light transmission and depth of cure.
The value of polymerization shrinkage was slightly dependent of colour in the case of FC composites. In details, it was almost exactly an inverse relation in polymerization depth. Differences statistically significant occurred in the case of yellow and FA A1, violet, blue and green composite as well as pink/violet and pink/orange.

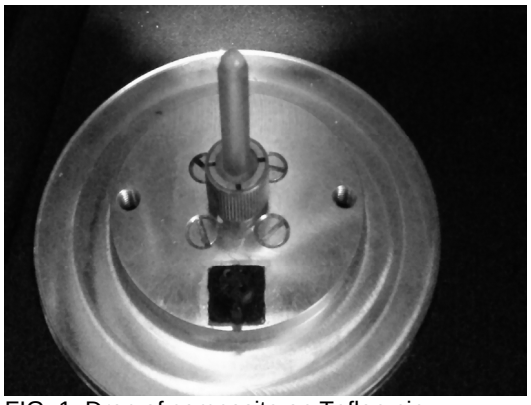

FIG. 1. Drop of composite on Teflon pin mounted on CT stage.

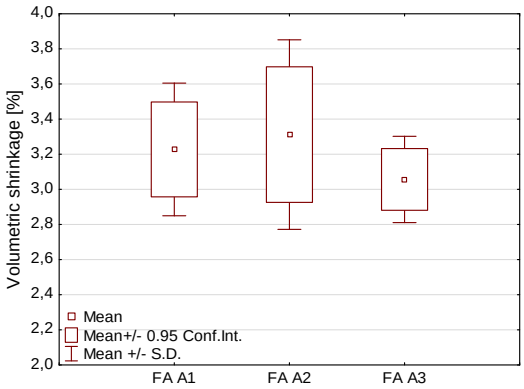

FIG. 2. Polymerization shrinkage of FA composites.

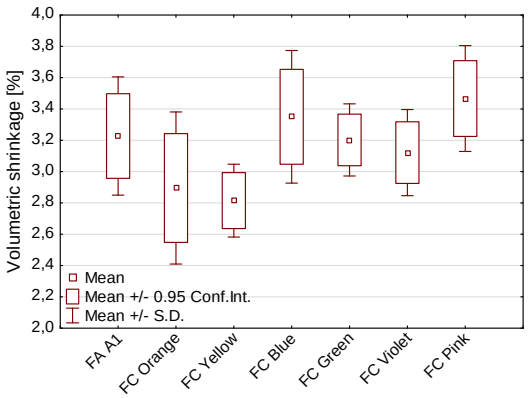

FIG. 3. Polymerization shrinkage of FC composites vs. FA material.

Conclusions

All tested materials showed low value of polymerization shrinkage, comparable with other commercial flow-type composites. The relation between depth of cure and the polymerization shrinkage was demonstrated.

Acknowledgments

Authors acknowledge ARKONA Laboratorium Farmakologii Stomatologicznej for sharing materials to the tests.

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BIOCOMPATIBILITY NORMATIVE INVESTIGATION OF NEW CO-POLYMER INTENDED FOR MANUFACTURING OF VENTRICULAR ASSIST DEVICES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 76]

Introduction

Nowadays one of the "gold standard" of end stage heart failure treatment is mechanical circulatory supporting by means of ventricular assist devices (VAD). Pulsating VAD (for adults as well as for children) are made of biocompatible polymers resistant to biodegradation. As a part of the Polish Artificial Heart project [1] the new biocompatible co-polymer intended for Polish pulsatile VADs has been developed.

The goal of presented study was to assess the biocompatibility of developed co-polymer according to regulations mandatory for medical products.

Materials and Methods

The investigated material was poly(aliphatic/aromatic-ester)s (PED) containing hard segments as in poly(ethylene terephthalate) (PET) and soft segments of dimer of linoleic acid (DLA) [2]. The weight percentage content of PET was 65% (hereinafter called PET65) or 70% (called PET70). All tested samples were manufactured of granulate by means of injection moulding and sterilized by radiation (25kGy).

Investigation was carried out according to following sections of PN EN ISO 10993 standard:

s.4: hemolysis: investigation was carried out on human whole blood preserved by means of CPDA-1. Based on plasma free haemoglobin concentration the index of hemolysis (IH) was calculated. Test duration: 8 and 24h.

s.5: cytotoxicity: examinations were carried out on fibroblasts L929 incubated for 24h in Medium 199 supplemented by 10%FCS. Live and necrotic cells were marked by FDA and PI, respectively.

s.6: local effect after implantation: reference biomaterials for PET65 and PET70 were Bionate90A and Bionate55D (DSM Biomedical, USA), respectively. Apiece 5 rabbits were implanted for each biomaterial and for blind test (surgery only, no implant). Observation duration: 4 and 12 weeks. Assessment of AlAT, AspAT, bilirubin, CRP, IL6, C5a, blood morphology and organs histopathological examination after euthanasia was performed.

s.9: biodegradation: biodegradation tests were being carried out for 30, 60 and 180 days in SBF. Following aspects were assessed: matter eluviation to SBF (HPLC), polymer degradation (GPC), chemical and morphological surface degradation (FTIR and SEM, respectively) and glass transition temperature (DSC).

s.10: intradermal reactivity: intradermal injection of PET65 and PET70 extracts in sesame oil and 0.9%NaCl was carried out. As a reference pure solvents were injected. Apiece 3 rabbits per each biomaterial were utilized. Duration of animals' observation: 24, 48 and 72 hours. Assessment of erythema, eschar and oedema according to scoring scale attached in standard was done.

s.10: allergic reaction: examination were carried out in accordance with GPMT test. PET65 and PET70 extracts in acetone solvent as well as pure acetone (as a reference) were used. Apiece 10 and 5 guinea pigs were utilized in tested and reference groups, respectively. The erythema was assessed according to Magnusson-Klingsman scale.

s.11: subacute systemic toxicity: intraperitoneal implantation of PET65 and PET70 (apiece 6 rabbits per one biomaterial and blind test). Observation duration: 28 days. Assessment of AlAT, AspAT, bilirubin, CRP, blood morphology and organs histopathological examination after euthanasia were performed.

s.11: acute systemic toxicity: intravenous injection of PET65 and PET70 extracts in 0.9%NaCl (apiece 3 rabbits per one biomaterial and blind test – pure 0.9%NaCl injection) was done. Observation of animals' behaviour per 7 days was carried out.

Results and Discussion

s.4: hemolysis: in all cases IH<0.5% (upper level=2.0%). Investigated materials are non-haemolytic.

s.5: cytotoxicity: no lysis as well as reduction of cells' growth were pointed out. The level of cytotoxicity of investigated materials is: no toxic.

s.6: local effect after implantation: all biochemical and morphological parameters were in physiological ranges. No statistically significant differences of parameters between tested and control group were pointed out ($p>0.50$). No significant changes in histopathological picture of organs as well as wound were shown.

s.9: biodegradation: no differences in GPC, HPLC and FTIR spectrums as well as SEM pictures acquired before and after degradation were shown. Investigated materials are high resistant for biodegradation.

s.10: intradermal reactivity: the scoring for PET65 and PET70 was 0.66 and 0.64, respectively. Scoring<1 denotes no intradermal reaction of investigated materials.

s.10: allergic reaction: in any case the scoring in Magnusson-Klingsman scale equalled zero. Investigated materials didn't cause allergic reaction.

s.11: subacute systemic toxicity: all biochemical and morphological parameters were in physiological ranges. No statistically significant differences of parameters before and after implantation were pointed out ($p<0.05$). No significant changes in histopathological picture of organs as well as wound were shown.

s.11: acute systemic toxicity: no changes in animals' behaving, weight and site of injection were found.

Conclusions

Normative investigation carried out according to ISO10993 demonstrated, that PET65 and PET70 are non-haemolytic, non-toxic, no allergenic and strongly resistant to biodegradation. High level of biocompatibility makes those materials suitable to application in medical devices. It is recommended to carry out additional investigation concerned the thrombogenicity and industrial processing of designed co-polymer.

Acknowledgments

This work has been financially supported by the Polish Artificial Heart project and by the National Centre of Research and Development (PBS/A5/2/2012).

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SELECTED IN-VITRO STUDY OF BIONANOCELLULOSE TOWARD ITS UTILIZATION IN MEDICAL DEVICES CONTACTING WITH BLOOD

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[ENGINEERING OF BIOMATERIALS 138 (2016) 77]

Introduction

Prospectively, bionanocellulose (BNC) can be a widely applied biomaterial e.g. for manufacturing of dressings, contact lenses and implants like cartilages, valves or pericardial patches. BNC is a product of fermentation carried out by bacteria *Gluconacetobacter xylinus E25,* Cellulosic fibbers in diameter ap. 100um are cross-linked in the form of 3D nano-structure.

The goal of study was to assess the possibility of BNC application as a material to manufacturing medical devices permanently contacting with blood.

Materials and Methods

BNC was manufactured by Bowil Biotech company [1]. Raw material was delivered in the form of 10cm square patches of thickness about 1mm. Samples were lubricated by 0.9%NaCl and tightly wrapped.

Investigation of BNC focused on in-vitro biocompatibility study of raw material as well as assessment of functional properties of BNC in respect of its application in vascular grafts.

Concerning the biocompatibility study the following tests were carried out:

1) biodegradation test was carried out according to ISO10993-9 and -13 standard in formulation buffer pH=6.54 containing lysozyme with concentration of 2mg/ml (40000U/mg). Incubation was carried out by 30, 60 and 180 days in temperature of 37^{o}C. The degradation of material during incubation was assessed by means of TGA/DSC method and compared with reference material (no exposed to lysozyme). The morphology of surface was assessed by means of SEM (80Pa, 10kV).

2) thrombogenicity test was carried out according to own examination method based on Impact-R test and apparatus [2]. The idea of Impact-R is to assess the platelet (PLT) function under physiological-like shear stress generated by rotating cone. The 14.4mm of diameter BNC samples were contacted with 130ul of fresh human citrated blood for 300sec. at 720RPM of cone rotational speed. After circulation the evaluation of PLT activation and aggregation were assessed. Leucocytes and platelets were marked by utilizing CD45, CD62P and CD61 antibodies. Activated cells were measured by means of cytometry. Cells adhered to the surface of BNC samples were examined by means of fluorescent microscope. The reference was polystyrene. The positive control was polystyrene covered by Bionate 2 (manufactured by DSM) with manually rugged surface. Data were processed by means of ANOVA Kruskal-Wallis test and Mann-Whitney U-test.

Concerning the study of functional properties of BNC following tests were carried out:

3) permeability of BNC patches was assessed according to ISO 7198 standard on pure water and on porcine blood. Additionally, the permeability of commercial sealed graft (Gelveave/Vascutech and Intergard Knitted Graft sealed by means of gel and collagen, respectively) as well as unsealed graft (Bard type) was measured. Data were processed by means of ANOVA Kruskal-Wallis test and Mann-Whitney U-test.

4) athrombogenic features of grafts (3/8'' in diameter, 9cm of length,) made of BNC was examined based on acute thrombogenicity test [3] carried out on porcine blood anticoagulated by means of heparin. This qualitative test shows is device made of investigated biomaterial tend to form and adhere thrombus under continuous and pulsatile blood flow.

Results and Discussion

1) slightly decreasing of BNC thermal stability was observed which is related to formulation buffer migration insight the BNC fibre matrix. This thesis was confirmed in SEM examination with demonstrated buffer crystallites bounded to samples surface. In spite of minor decreasing of BNC thermal stability the material itself is resistant to biodegradation in lysozyme.

2) related the PLT activation there was no statistically signifficant differences between BNC, reference and positive control ($p>0.3299$). Related the PLT adhesion the comparison of pictures of samples revealed no differences between BNC and reference. The surface of positive controls was covered by numerous groups of aggregates. In respect of thrombogenicity BNC is equivalente to polystyrene.

3) mean permeability of BNC, gel-sealed, collagen-sealed and reference grafts in test carried out on blood were 0, 0, 2 and 26 ml/min/cm^2. In test carried out on water results were 0, 0, 5 and 196 ml/min/cm^2, respectively. Performed test showed, that BNC is impermeable for water as well as for blood.

4) in all cases spontaneous decreasing of ACT were occurred from ap. 260 s after heparin administration to ap. 140 s (end point of circulation). In two cases BNC surface contacted with flowing blood was free of visible clots formation. Immunohisochemical examination (eosin & haematoxylin dyeing) revealed insignificant fibrin layer with loosely bounded blood cells. In third case larger clots were formed as a result of ridged BNC surface. In all cases no hemolysis was found.

Conclusions

The BNC is resistant for enzymatic biodegradation. Its thrombogenicity is comparable to polystyrene. Material itself is impermeable for blood as well as for water with makes it suitable for manufacturing grafts and patches permanently contacted with blood. Additional examinations (e.g. animal trials) are necessary to confirm athrombogenic features of grafts made of BNC. It is essential to assess mechanical properties of BNC paying special attention to potential sphere of its application.

Acknowledgments

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CYTOTOXIC SURFACES FOR REGIONAL CHEMOTHERAPY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 78]

Introduction

Regional chemotherapy, in contrast to conventional approaches, enables local delivery of anti-cancer compounds limitting the side-effects of the treatment [1]. Among a wide range of possible drug carriers, conducting polymers are unique materials allowing highly controlled, electrostatic immobilization and release of a variety of biologically active compounds (FIG. 1). One of the most stable polymers exhibiting biocompatibility and high conductivity is PEDOT – this is why this polymer was selected to serve as a platform for the immobilization of betulin (FIG. 2) – one of the most often occurring triterpene presenting a broad range of biological properties, i.e. anti-cancer, anti-bacterial, anti-inflammatory, anti-fugal and anti-viral activities [2].

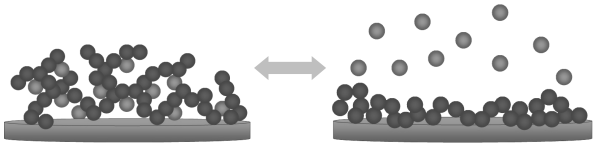

FIG. 1. The schematic representation of ion-exchange properties of conducting polymers.

Materials and Methods

The process of drug incorporation was performed by means of the three-step procedure that is described in details in [3]. In this procedure, the monomer is electrochemically polymerized in the presence of the primary dopant, then the doping ions are removed from the polymer matrix via application of reduction potential, and drug is immobilized via the re-oxidation of the matrix in the presence of drug. The drug-loaded PEDOT matrix was characterized by means of Raman and IR spectroscopies, as well as SEM. The process of drug release was triggered by means of cyclic voltammetry, and the cytotoxic activity of released drug was verified against KB and MCF-7 cancer cell lines.

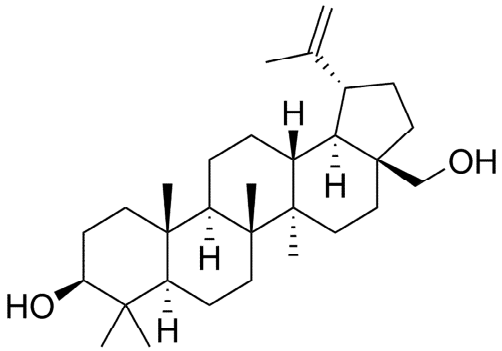

FIG. 2. Chemical structure of betulin.

Results and Discussion

The proposed immobilization procedure was proven to be an efficient method of drug incorporation resulting in the formation of betulin/PEDOT composite. The release of betulin immobilised in conjugated polymer matrix was performed under spontaneous (passive) and electro-assisted (active) modes in PBS. The range of applied reduction potentials was optimized – low enough to start the process of release but not too low, to prevent the process of matrix degradation.

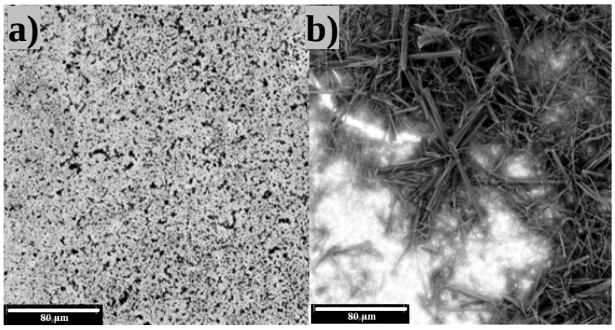

FIG. 3. SEM images of PEDOT (a) and betulin/PEDOT (b) matrix.

In vitro studies conducted with human oral carcinoma (KB) and human breast adenocarcinoma (MCF-7) cancer cell lines showed that for both types of cancer cell lines the solutions obtained as a result of active release exhibited the lowest IC_{50} values (13.34 ± 0.88 µg/ml and 12.57 ± 1.81 µg/ml for KB and MCF-7, respectively), hence they possessed the highest cytotoxic activity among all investigated samples. For comparison, the IC_{50} values for the samples released by means of spontaneous mode were equal to 19.25 ± 0.15 µg/ml and 20.05 ± 3.12 µg/ml for KB and MCF-7, respectively.

Conclusions

Betulin/PEDOT composite was shown to be a promising material for the surface modification for the needs of regional chemotherapy. The IC_{50} values of released drug were found to be comparable with the results of cytotoxic activity of betulinic acid against MCF-7 cancer cell lines [4], showing that the processes of electrochemical drug immobilization and release had no adverse effects on its biological activity.

Acknowledgments

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POLY(3,4-ETHYLENEDIOXYPYRROLE) – NOVEL CONDUCTING POLYMER WITH BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 79]

Introduction

Biomedical engineering requires constant development of new types of biomaterials with specific properties. Conducting polymers have found to be promising materials applicable in the field of biosensors, artificial scaffolds and neural probes [1]. Nevertheless, only several conducting polymers exhibit biocompatibility and stability which are properties necessary for such type of applications. Poly(3,4-ethylenedioxypyrrole), PEDOP (FIG. 1), is a novel conducting polymer which is the most promising candidate to become an alternative for polypyrrole and poly(3,4-ethylnedioxythiophene) in a field of electroactive biomaterials [2,3].

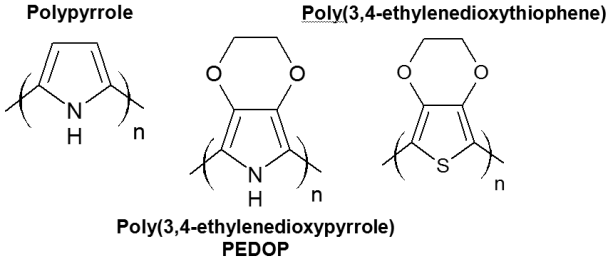

FIG. 1. Chemical structures of conducting polymers applied in biomedical engineering.

In this study, the description of physicochemical properties of PEDOP matrix is presented, involving electrochemical, spectroscopic and microscopic analysis. PEDOP is also used as a drug carrier for model drugs – ibuprofen, quercetin and ciprofloxacin.

Materials and Methods

The process of drug immobilization was realized with the use of electrochemical techniques, i.e. cyclic voltammetry and chronoamperometry. The efficiency of drug immobilization was studied by means of UV/Vis spectrophotometry. Raman spectroscopy and scanning electron microscopy were used to analyze structural and surface properties of polymer matrices, while electron paramagnetic resonance data allowed to follow the changes in spin concentration resulting from reduction-oxidation processes.

Results and Discussion

The voltammetric studies on EDOP showed that this monomer can be oxidized at very low potential (Eox = 0.7 V), substantially lower than for EDOT (Eox = 1.0 V) and similar to pyrrole (Eox = 0.7 V). This indicated that the process of drug immobilization can be carried out under mild conditions, not destructive for drug molecules. The highly controlled, regular growth of polymer, as well as its substantial stability, were proven by means of UV-Vis spectroelectrochemical studies.

The immobilization of drugs was performed via performing polymerization procedure in the presence of drug molecules. Due to the possibility to control the growth of polymer film, PEDOP matrices of different thicknesses (obtained via different number of CV cycles) were synthesized and used for the release experiments. FIG. 2 shows how the choice of drug and matrix thickness influenced the drug-loading efficiency of PEDOP, as well as the ratio of passive to active release modes.

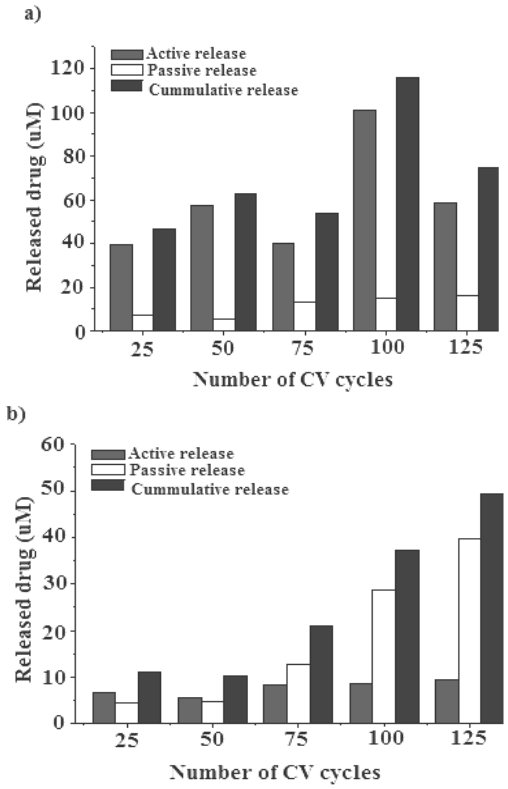

FIG. 2. The amount of ciprofloxacin (a) and quercetin (b) released from PEDOP matrix as a function of matrix thickness expressed in the number of CV cycles.

Conclusions

The physicochemical properties of PEDOP indicated this polymer as being the favourable among conventional materials, i.e. polypyrrole and PEDOT. The high drug loading efficiency of PEDOP and the possibility to immobilize a variety of biologically active compounds proved that it is advantageous drug carrier and can be used as the matrix for controlled drug delivery systems.

Acknowledgments

The authors would like to thank the Polish National Science Centre (PRELUDIUM, 2012/07/N/ST5/01878) for financial support.

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PHASE TRANSITION OF CHITOSAN CHLORIDE SOLUTIONS AS POTENTIAL MATERIAL FOR APPLICATION IN BIOMEDICAL ENGINEERING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 80]

Introduction

Chitosan is a semi-crystalline amino-polysaccharide obtained through deacetylation of chitin. Due to its physicochemical properties, chitosan has a variety of applications in sewage treatment processes (chelating properties), food industry and biomedicine – in processes of creating smart drug carriers [1].
Chitosan is dissolved in low concentrated solutions of organic and non-organic acids in which it forms salts with dissolvent ions. Chitosan properties change from hydrophobic to hydrophilic and a colloidal system is created in which the polymer chains remain dispersed in a continuous medium – acid solution. Such a system shows a possibility for a sol-gel phase transition. Chitosan hydrogels may form as a result of increasing the concentration of the polymer [2], through the change of pH of the solution or through heating it [1,3,4].
The aim of this work was to create hydrogels sensitive to temperature changes from low-concentration solutions of chitosan and to describe the phase transition by determining the gelation point and activation energy.

Materials and Methods

For the research on chitosan hydrogels three types of chitosan were used. Two high-viscous from the Sigma-Aldrich company (chitosan from shrimps and crabs) and a low-viscous chitosan from the Fluka company. Aqueous solution of hydrochloric acid was used as a dissolvent. β-Glycerophosphate disodium (β-NaGP) was used as a buffer.
The chitosan solution (2.5% w/v) was prepared by dissolving 0.4 g chitosan in 16 ml 0.1M HCl solution. After dissolving, the container with the sample was covered and left for 24 h in room temperature. After that time the sample was left in temperature 5°C for 2 h. Two solutions were made for each kind of chitosan. A previously prepared solution of 2g β-NaGP in 2 ml distilled water was added to one of them, whereas the other solution was left without β-NaGP.
The measurements of rheological properties were conducted in a cone-plate system of a rotational rheometer Anton Paar Physica MR 301. A cone of 50 mm diameter and 1° angle was used. The gelation process was conducted in non-isothermal conditions. Chitosan solutions were put in 5°C temperature in the measuring system of a rheometer. Next the samples were heated with a constant speed of 1°C/min. The phase transition temperature was determined by crossover point of storage modulus G' and loss modulus G'' curves.

Results and Discussion

Based on measurements, the evolution of storage modulus G' and loss modulus G'' as functions of temperature were obtained. From the course of the G' G'' curves, three characteristic regions can be observed. In first region, the chitosan samples show a typical behaviour for a viscoelastic liquid. There is a predominance of loss modulus over storage modulus. In second region, the values of the storage modulus and the loss modulus rise rapidly. This is a result of the creation of a crosslinked structure (gel). Domination of the storage modulus over the loss modulus was also observed. In the final third region, the gelation process proceeds more slowly. This results from large viscosity values of the medium, slowing down the diffusion of the molecules. The character of the arisen structure changes to glass form.
Comparison of chitosan solutions containing β-NaGP and without this addition reveals that solutions with higher pH value show lower gelation temperature (close to physiological temperature of human). Another basic difference in the gelation phenomenon between the two types of solutions is revealed in the dynamics of changes in second region. A more rapid change for solutions without the addition of β-NaGP compared with the solutions containing the buffer can be observed.
Kinetic model of polymer crystallization [5] allows to determine the activation energy for the gelation process. Comparison of chitosan solutions samples of the same type in two versions (with and without the addition of β-NaGP) indicates that the formation of the structure is more advantageous energetically for solutions with the addition of buffer. The determined value of activation energy also indicates that second region is the key area in the gelation process, absorbing the most input heat energy.

Conclusions

The sol-gel phase transition of chitosans chloride while heated with constant speed of temperature increase is performed in three characteristic areas: (1) the solutions exhibit the behaviours of a viscoelastic liquid, (2) the process of fast crosslinking close to the gelation point – creation of a soft rubber structure, (3) the process of slow gelation at high temperatures – formation of a glass structure.
It was stated that chitosan chloride solutions with β-NaGP until the sol-gel phase transition require far lower energy than analogous systems without β-NaGP.
From the viewpoint of medical use, the systems with the addition of β-NaGP are more desirable. The phase transition temperatures of these systems are close to the human body temperature (~37°C) as opposed to systems without the addition of buffer where the phase transition point drastically exceeds that temperature.

Acknowledgments

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CARBON NANOPOWDERS IN TECHNOLOGY DRUG FORM

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[ENGINEERING OF BIOMATERIALS 138 (2016) 81]

Carbon nanopowders can be a perfect delivery vehicles for macromolecular biotechnology products due to their reactive surface and exceptional biocompatibility [1]. These nanomaterials may transport the active substances directly to the target place in the body affect its distribution or prolong its duration of action.
The small size of nanoparticles allows to pass through the smallest blood vessels, thereby improving the effectiveness of therapy. Moreover, for the same nanopowders have been documented anti-inflammatory properties. Thanks to its excellent biological properties, which include high biocompatibility and antioxidant properties, carbon nanopowders are the subject of research in many centers around the world. Thanks to the high reactivity of carbon nanopowders, associated with the presence of dangling bonds on the surface, there is a broad spectrum of possibilities of connecting a number of functional groups that are adequate in terms of further treatment processes, directed to obtain a final functionalized product [2]. Recent work on carbon nanopowders are now devoted to the possibilities of their use in chemotherapy, as anticancer delivery vehicles. As antioxidants it can be used to treat other diseases, resulting as the effect of free radicals, such as Alzheimer's disease, Parkinson's, atherosclerosis, diabetes, bronchial asthma or rheumatoid arthritis [3]. This subject is still in early stages of research and needs further study**.**

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ANODIZED TiO_2 COATINGS RESISTANT TO MICROBIAL COLONIZATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 82]

Introduction

In the recent development of biomedical engineering and biomaterials, there was observed an increasing need for the manufacturing of materials that would not only replace or heal the damaged parts of human body, but also block the microbial colonisation. Development of the way of how to inhibit the implants microbial colonisation has been considered as one of the very important aspects in biomaterials surface engineering. Among many approaches that allow to decrease adhesion of bacteria or fungi to materials there are protective coatings (diamond-like carbon, titanium dioxide etc.), immobilization of antimicrobial agents and particles onto surfaces and many others. Literature findings focus extensively on the use of TiO_2 layers for antimicrobial protection [1,2].

One of the popular, easy-to-implement and fast manufacturing techniques for TiO_2 is anodic oxidation. Anodization can be performed in various electrolytes, under desired voltages and currents. The change in the process parameters can lead to the creation of different oxide layers that could be porous, structurized or compact [3-5]. To obtain structurized TiO_2 coatings fluoride-based electrolytes can be used. These types of layers are known to possess excellent biological properties (e.g. increased osteoblast proliferation) [6]. However, they usually are not tested for microbial colonisation.

This work focuses on the dependence of the process parameters during titanium anodization on creation of structurized TiO_2 films and assessment of microbial colonization on their surfaces.

Materials and Methods

Substrates being titanium alloy Ti6Al4V disks were subjected to anodization in water-based electrolytes containing hydrofluoric acid as the oxidizing agent. Samples were manufactured with different approaches:

a) Different deposition times (10, 20 and 60 minutes)
b) Different deposition voltages (10, 20 and 100 V)
c) Changing the amount of hydrofluoric acid (0.25, 0.5, 1, 1.5 and 2 vol.% of the electrolyte)

The surface characterization based on scanning electron microscopy was performed. For the evaluation of microbial colonisation, bacterial (*Escherichia coli*) and fungal (*Candida albicans*) strains were used.

Results and Discussion

The topographical examination of prepared coatings showed that with the changing deposition parameters, there are changes in the complexity and structurization of coatings. The nearly-tubular structures that are the most desired for tissue regeneration near implant were obtained for titanium anodization in 2% vol. HF electrolyte.

The higher was the structurization of the coating, the higher was also the microbial colonisation. Not only the highest number of live bacteria cells were attached to surfaces possessing small structurization, but also almost a linear increase of total area occupied by bacteria with the increase of structurization is observed.

In the case of colonisation by *Candida albicans*, the situation is slightly opposite. The fungal attachment on surfaces is the smallest for the most complex TiO_2 coatings.

In both cases, all anodized samples exhibited the anti-microbial character due to lower attachment of bacterial and fungal cells in comparison to the control sample.

Conclusions

This study showed that the bacterial and fungal adhesion can be modulated by means of simple surface structurization. Not only the shape of the irregularities, but also the size of cells willing to inhabit those surfaces is important. What is more, the higher is the microbial colonisation of surface, the reduced could be the proliferation of human cells like e.g. osteoblasts. Thus, further modifications to manufactured surfaces like doping may be needed in order to reduce the possibility of microbial biofilm formation on anodized surfaces.

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TITANIUM ANODIZATION IN ETHYLENE GLYCOL-BASED ELECTROLYTES FOR SURFACE STRUCTURIZATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 83]

Introduction

Anodization of titanium is a very popular technique used in order to obtain the titanium dioxide protective coatings [1]. Among many electrolytes that can be used to obtain different structures and properties of TiO_2, ethylene glycol based solutions with the addition of water and fluoride-containing salts or acids can be used for the development of nano-tubular and nano-structurized coatings, among which TiO_2 nanotubes arrays are one of the most common. Organic additive to the electrolyte slows the dissolution of the formed oxide and thus, the more regular structures are created [2-4].
This work is devoted to evaluate the dependence of the different ethylene glycol based electrolytes on structure and the antimicrobial properties of anodized titanium.

Materials and Methods

Ti6Al4V disks of d=16 mm were mechanically polished and subjected to the anodization in solutions with different water to ethylene glycol ratios. All electrolytes were containing 2% vol. of hydrofluoric acid. Samples were deposited in constant voltage of 20 V and the deposition time was 20 minutes.
Scanning electron microscopy was used for surface complexity evaluation. What is more, roughness of the samples to evaluate the level of surface structurization was investigated. What is more, the bacterial (*Escherichia coli*) and fungal (*Candida albicans*) adhesion to the anodized surfaces was evaluated.

Results and Discussion

The water to ethylene glycol ratios were 10:90, 20:80, 30:70, 40:60 and 50:50, respectively. The scanning electron microscopy evaluation revealed that the highest structurization was obtained for sample being anodized in the solution of 70% vol. of ethylene glycol. When there was about 90% vol. of ethylene glycol in the solution, the surface changed its character from structurized to micro-porous. Also, the porous surfaces had an average roughness Ra being almost 3 times higher than for structurized samples.
Bacterial colonisation on manufactured surfaces showed almost the linear growth of the total area occupied by bacteria in comparison to control sample when ethylene glycol to water ratio was increased. However, for *Candida albicans* there is no linear dependence between the electrolyte composition and fungal surface colonisation. In this case, almost for all samples the number of cells occupying the surface was similar, with the only exception for sample deposited in electrolyte with water to ethylene glycol ratio being 40:60 – here the number of *Candida* cells attached was much higher in comparison to others.

Conclusions

The study showed that the anodization of titanium is possible by means of use of organic electrolytes. However, as it is known that the reduction of water may cause the help of formation of more tubular, longer structures of the coating, our study has not showed that dependence. For each of the sample, different surface character and structure was obtained. However, all the samples exhibited the anti-adhesive character for the purpose of inhibiting the bacterial and fungal biofilm development.

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POLYOXYMETHYLENE HOMO- AND COPOLYMER COMPOSITES STABILIZED WITH PEG-GRAFTED HYDROXYAPATITE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 84]

Introduction

Polyoxymethylene (POM) is a highly crystalline thermoplastic polymer. POM is mostly known for its high mechanical strength, stiffness, low friction coefficient, good chemical resistance and low water absorption [1]. There are two different kinds of polyoxymethylene, depending on production method: POM homopolymer (POM_H) and POM copolymer (POM_C). POM homopolymer crystallizes very easy and has excellent mechanical properties [2]. The main disadvantage of POM_H is its very high thermal sensitivity – autocatalytic decomposition of polymer chains occurs at elevated temperatures [3]. POM copolymer (POM_C) is produced by cationic polymerization of trioxane with the presence of comonomers, such as ethylene oxide or dioxane. It has slightly worse mechanical properties than POM_H, but its thermal resistance is higher [4].

POM has been used in heart valves replacements and orthopedic implants such as hip and knee prosthesis for years. Furthermore, POM is used in dentistry as a substitute of metals and acrylic resins in numerous prosthesis applications [5]. Now, it is proposed as a material for bone long-term implants.

Initially, POM/hydroxyapatite (HAp) nanocomposites were obtained [6]. Unfortunately, thermal stability of these composites decreased significantly even by 30°C. In our previous study, we modified HAp with poly(ethylene glycol) (PEG) 2000 (HAp-*g*-PEG 2000) and introduced into the POM copolymer matrix in order to improve thermal stability of POM [7]. In this work the influence of HAp-*g*-PEG 600 on the thermal stability of POM_H and POM_C is investigated.

Materials and Methods

Ultraform® POM copolymer (BASF, Germany) and Delrin® POM homopolymer (DuPont™, USA) were used in this work. Stoichiometric HAp nanopowder was product of nGimat Co (Atlanta, USA). 1,6-hexamethylene diisocyanate (HDI), dibutyltin dilaurate (DBTDL) and poly(ethylene glycol) (PEG) with average molar mass 600 were supplied by Sigma Aldrich. Anhydrous DMF and ethanol were products of Avantor (Poland).

Grafting process

Firstly, the HAp-*g*-PEG 600 filler was prepared: 9 g of HAp was dispersed in 90 ml of dry DMF. Next, 9 µl of DBTDL catalyst was introduced to HAp dispersion. Then, the solution of 6 ml HDI in 12 ml DMF was dropped to HAp. After that, the temperature was increased to 78°C and kept for 2.5h. In the second step, the mixture was cooled down to 40°C, 10.8 g PEG 600 was dissolved in DMF (1:1 w/v) and dropped to the suspension. The temperature was increased to 65°C and the mixture was stirred for 1.5 h under nitrogen. At the end, the powder was separated in centrifugal separator and washed three times with ethanol. After that, the HAp-*g*-PEG 600 powder was dried at 40°C for 24 h.

Processing

Two types of POM (POM_C and POM_H) was modified in this study. POM and POM/HAp-*g*-PEG 600 composites were prepared by melt processing methods. In the first stage, POM and HAp-*g*-PEG 600 powder were mechanically mixed (0, 0.5, 1.0, 2.5, 5.0 and 10.0% w/w of HAp-*g*-PEG 600) (calculated in relation to pure HAp) and extruded in a twin-screw extruder (50 rpm, 210°C). Compositions were then shaped by injection moulding method.

Thermal analysis techniques, such as DSC and TG, were used to characterise the obtained composites. FTIR spectroscopy and SEM microscopy were performed as well. Mechanical tests, in the tensile mode, were also conducted. The formaldehyde release during incubation was analysed using Schiff's reagent.

Results and Discussion

Thermal stability of POM_C and POM_C/HAp-*g*-PEG 600 composite was investigated with TG method (FIG. 1).

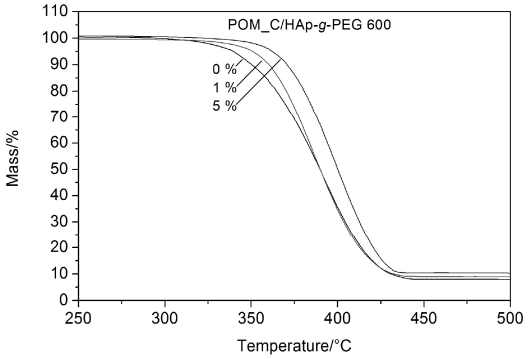

FIG. 1. TG curves of pure POM (0%) and POM/HAp-*g*-PEG 600 composites (1%, 5%).

HAp-*g*-PEG 600 contributed to the increase of thermal stability of POM from 315°C (pure POM) to 343°C (5% HAp-*g*-PEG 600). This effect can be explained by the decrease of the quantity of free hydroxyl groups in HAp which are able to catalyse reactions [8]. The presence of nitrogen atoms in the urethane bonds can also increase the stability of POM, as nitrogen-containing compounds such as polyamides and dicyandiamines are usually used as a heat stabilizer of POM [9]. In comparison to our previous study, the molar mass of PEG does not affect the efficiency of HAp-g-PEG thermal stabilizer.

Conclusions

The present results confirmed that incorporation of HAp-*g*-PEG 600 in POM matrix causes a considerable increase in POM thermal stability. Thereby, the risk of the deterioration of material properties after the polymer processing at elevated temperatures is minimized, which is crucial in the use for orthopedic applications.

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PREPARATION AND CHARACTERIZATION OF POROUS COMPOSITES BASED ON POLYURETHANES AND BIOCERAMICS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 85]

Introduction

One of many challenges in modern materials science is to find new solutions for implantology, which would efficiently facilitate and accelerate bone regeneration. Biomimetics is common approach, which takes into account composite structure of bone tissue. In the process of designing bone implants, materials with similar chemical composition and mechanical properties as these of a bone, are used. Such materials are biocompatible ceramics (hydroxyapatite and TCP) and polyurethane, and both of them were used in experimental stage of this paper [1,2].

Materials and Methods

The objective of work was to develop synthesis method and to characterize selected properties of polyurethanes modified with bioactive ceramics (TCP, HAp), and to perform preliminary assessment of the possibility of their use in orthopaedics [3,4].

Two series of polyurethane/ceramics (β-TCP or HAp) composites were synthesized by one stage bulk polymerization method. Next, FTIR and DSC analyses were performed, as well as mechanical properties, porosity, surface analysis after incubation in SBF and chemical stability in water environment were investigated.

Results and Discussion

Spectroscopic analysis confirmed the lack of linkages between the polyurethane matrix and ceramic additives and complete conversion of reactants. DSC analysis showed the presence of a glass transition of soft segments - glass transition temperature shifted to higher temperatures with the addition of hydroxyapatite. Mechanical tests have shown that both additives result in improved mechanical properties. Porosity test results showed that the best distribution of pores occur in PU composites modified by hydroxyapatite. Preliminary assessment of bioactivity showed that only the addition of 20% β-TCP allowed the accretion of apatite on the material. In the case of additive which is hydroxyapatite, bioactive properties occur in the PU materials containing 2.5%, 5% and 10% Hap.

Conclusions

In this work porous polyurethane based materials modified with bioactive ceramics were obtained. Based on preliminary results it can be concluded that the presented materials have potential for the regeneration of bone tissue.

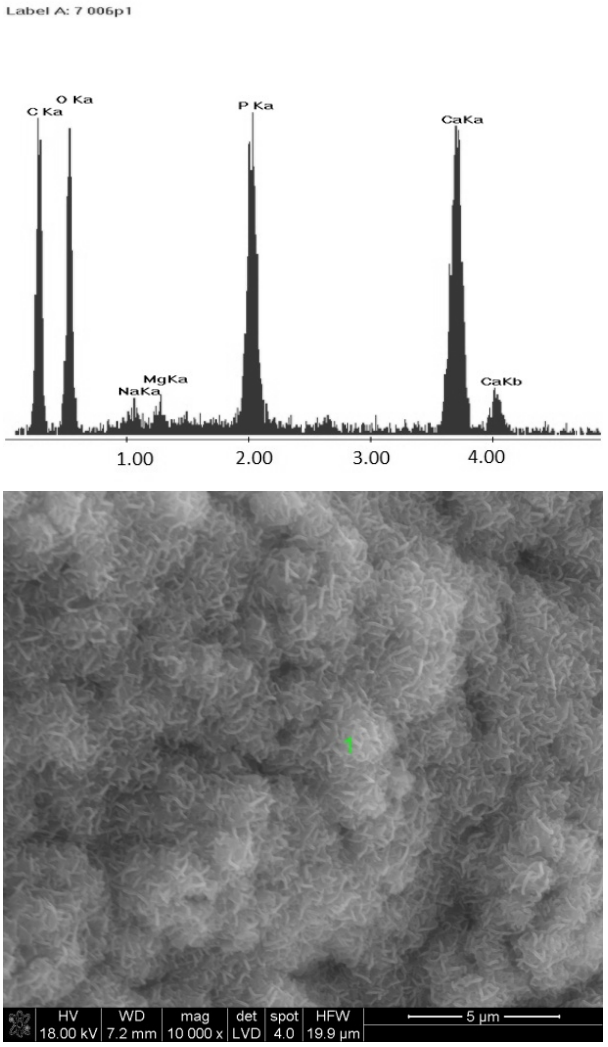

FIG. 1. SEM microphotographs and EDS results for PU composite with 5% HAp after incubation in SBF.

Acknowledgments

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OSTEOINDUCTIVE PROPERTIES OF GEL-DERIVED BINARY $CaO-SiO_2$ AND TERNARY $CaO-P_2O_5-SiO_2$ BIOACTIVE GLASSES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 86]

Introduction

Bioactive glasses have been long recognized for their ability to integrate well with bone tissue and support bone tissue formation and remodelling. However, the emerging new feature of this biomaterial group is the ability of some bioactive glasses to induce osteogenic differentiation of mesenchymal precursor cells *in vitro* and/or *in vivo* without the necessity of additional cell treatment with osteogenic growth factors. Such osteoinductive material properties are promising for the clinics as their implantation would not require supplemental treatment with osteogenic compounds. In this work we have examined osteoinductive properties of PLGA-based composites containing gel-derived bioactive glasses differing in their $CaO:SiO_2$ ratio. They were used as growth surfaces for human BMSC cultures that were not treated by any other, but materials, osteogenic compounds. Cells were examined at different culture times for the expression of osteogenic growth factors (BMP-2), early osteogenic transcription factors (Runx-2 and Osterix), alkaline phosphatase (ALP) activity and extracellular matrix mineralization. The direct effects of surface chemistry or the ions released to the culture medium due to material surface activity were evaluated for their osteoinductive properties. Also, the materials were pre-incubated in simulated body fluid (SBF) before cell culture to compare cell responses with those cultured on "as-prepared" materials.

Materials and Methods

Bioactive glasses from the binary $CaO-SiO_2$ and ternary $CaO-P_2O_5-SiO_2$ systems were obtained by the sol-gel process (SBG, gel-derived bioactive glasses) and incorporated into PLGA basically as described by Zagrajczuk et al. [1]. TABLE 1 lists all compositions of SBGs examined in this study. The thin SBG-PLGA films were then sterilized by 70% ethanol and UV, and fitted into 24-well culture plates. Human BMSC were obtained from iliac crest of adult patients and, after expansion, cells were seeded directly on the studied material surfaces or they were cultured on standard tissue culture plastic and exposed to the culture medium previously used for the materials incubation. Some materials were pre-incubated in SBF for three days before cell culture. Human BMSC were examined for mRNA expression (real-time PCR) of BMP-2, Runx-2 and Osterix after 2 culture days, cell number (MTS assay) and ALP activity (biochemical kinetic assay) after 7 culture days and extracellular matrix mineralization (Alizarin Red S staining) after 14 culture days.

TABLE 1. PLGA-based composites containing SBGs of listed chemical composition [mole%].

Symbol of material	SiO_2	CaO	P_2O_5
A1	40	60	-
D1	60	40	-
T1	50	50	-
S1	80	20	-
A2	40	54	6
D2	60	36	4
T2	47	47	6
S2	80	16	4
PLGA-control	-	-	-
SiO_2-control	100	-	-

Results and Discussion

Culture analyses showed that practically each studied material induced osteogenic response of cells despite the cells were not treated with any osteogenic supplements. Increased calcium content in SBGs corresponded with increased BMP-2 mRNA levels and decreased Runx-2 levels, especially for binary SBGs. Notably, BMP-2 and Osterix mRNA levels were significantly higher in cells grown on substrates containing SBGs derived from ternary, P_2O_5 containing system, but ALP levels were comparable in cell cultured on substrates containing either binary or ternary SBGs. Preincubation of materials in SBF eliminated most of the observed differences in osteogenic response of cells, suggesting that the changes in the chemistry of scaffolds can significantly affect the response of cells, particularly if the materials are used "as prepared" without pre-incubation in SBF. We also found that the "ions extracts" collected from tested materials were capable to stimulate extracellular matrix mineralization in human BMSC cultures. This implies that the ions released from these materials on contact with cells and physiological fluids may play the key role in osteogenic cell responses.

Conclusions

Our studies suggest osteoinductive properties of gel-derived bioactive glasses derived from the binary $CaO-SiO_2$ and ternary $CaO-P_2O_5-SiO_2$ systems. The $CaO:SiO_2$ ratio in SBGs may influence the overall response of cells to SBG-containing materials and this is plausibly related to the ions released from these materials. It thus becomes possible to modulate cell responses depending on the SBG chemistry. On the other hand, incubation of materials in SBF before their exposure to the cells and tissues may change the biological outcome as SBF incubation moderates ions release and cell response.

Acknowledgments

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

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TWENTY YEARS OF RESEARCH ON GEL-DERIVED $CaO-P_2O_5-SiO_2$ BIOACTIVE GLASSES - WHAT NEXT?

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[ENGINEERING OF BIOMATERIALS 138 (2016) 87]

Introduction

Gel-derived bioactive glasses of $CaO-P_2O_5-SiO_2$ system have been developed by Laczka et al in 1995 year. The research was prompted by Li, Clark and Hench (1), who had reported by that time several advantages of gel-derived technology of bioactive glasses of $CaO-P_2O_5-SiO_2$ production.

Materials research

Three main gel-derived compositions were obtained by Laczka et al and compared with corresponding melted glasses:
A2 - $40SiO_2;54CaO;6P_2O_5$, T2 - $60SiO_2;36CaO;4P_2O_5$ and S2 – $80SiO_2;16CaO;4P_2O_5$ (mol%) (2-3). Since that time, these gel-derived bioactive glasses (SBG) have been studied in the form of homogenous sinters and coatings or incorporated into synthetic hydroxyapatite, ceramics (TiO_2) and polymers (PLGA and PCL) forming a composites (4-7). The latter were obtained either in the form of 2D films or 3D porous scaffolds. Produced materials were characterized with respect to chemical and phase compositions, microstructure, porosity, surface properties and ability of ions release to simulated body fluids. Moreover, bioactivity *in vitro* was estimate by simulated body fluid test. The ability to create a hydroxyapatite (HA) layer on the material surface was regarded as a sign of its biological activity and it was conditioned primarily chemical composition of biomaterials. Obtained results indicated that in the future it is appropriate to combine the *in vitro* SBF test with cell-basing experiments to a better evaluate the materials bioactivity.

Biological research

Both alone and as composite components these SBG-based materials have showed several osteogenic effects both *in vitro* and *in vivo*. Initially, SBG were examined for their immunological response in Wistar rats-derived macrophages, followed by rat osteogenic bone marrow cell cultures, later replaced by human bone marrow-derived mesenchymal stem cell osteogenic culture. Notably, despite different preparation forms, biological evaluations consistently showed some intrinsic osteogenic properties of these SBG materials, resulting in either their bone-forming (S2) or bone-remodelling ability (A2). Thus, we now come to conclusion that the chemical composition of these SBG materials plays primary role on contact with cells and tissues. The other SBG properties, such as amorphous/crystalline phase ratio, surface development and roughness, or material porosity do contribute to the overall osteogenic effects, but they play secondary role to these materials chemistry. Furthermore, the studies on T2 composition have been neglected for years due to initial results showing opposite to A2 and S2 immunological response, despite some promising data collected in rat bone marrow cultures. We now revaluate this T2 material as our most recent studies indicate that the moderate content of SiO_2 may be beneficial for early induction of osteogenesis by these materials in human bone marrow-derived cell cultures. Finally, all studied compositions belong to the bioactive group of materials, capable to form carbonate hydroxyapatite (HA) surface layer, which is believed to provide the prerequisite for bone tissue formation and integration. Despite this, most biological results have been obtained by us with “as-prepared” materials that were not pre-incubated to develop HA layer. Our recent studies with simulated body fluid pre-incubated materials indicate that the development of HA surface layer is beneficial for the overall osteogenic cell response, but it eliminates some key differences in the biological effects resulting from different material compositions. This correlates well with the gross amount of ions released from bioactive material surface before and after HA development. Altogether, the future applications of these and similar materials should focus on the material compositions, although examination of their different preparation forms is necessary, as the final product properties may either enhance or diminish the desired biological effects.

Acknowledgments

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

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PREPARATION OF FUNCTIONALIZED GRAPHENE OXIDE COATINGS ON GOLD

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[ENGINEERING OF BIOMATERIALS 138 (2016) 88]

Introduction

Gold has been well known as a metal that shows high biocompatibility. It has been applied for implantable medicine devices for instance to fabricate pacemakers and stents [1,2]. Moreover, it presents antibacterial properties as well [3]. Nevertheless, it has been reported that gold cannot be considered a perfect implantable metal since, unfortunately, it increases the risk of restenosis (reclosure of vessels in atherosclerosis due to interactions between metal stents and tissues) [4].

In the presented study we propose a coating of graphene oxide (GO) onto metallic gold surface. Such coating can isolate the surface of metal from direct contact with tissues and will preserve beneficial characteristics of gold such as current conductivity. Moreover, the functionalisation of GO by attachment of peptides or antibodies can lead to novel properties of the material such as active prevention from blood clotting. Also the introduction of functional groups can be useful in development of graphene based biosensors.

Materials and Methods

In the study a method of coating gold surfaces with GO which comprises binding GO to gold with the use of gold-sulphur bonds has been applied. For that purpose on a thoroughly cleaned surface of gold sputtered onto a glass slide, or a gold surface plasmon resonance sensing chip, a self-assembled monolayer (SAM) of compound containing thiol and amine groups (such as cysteine or cystamine) has been created. Subsequently, a GO layer has been connected to SAM by means of physical adsorption as described by Chiu et.al. [5]. Also, a well-known technique of amine coupling has been used for binding carboxyl groups of GO with amine groups in a two-step amine coupling process [6] (FIG. 1).

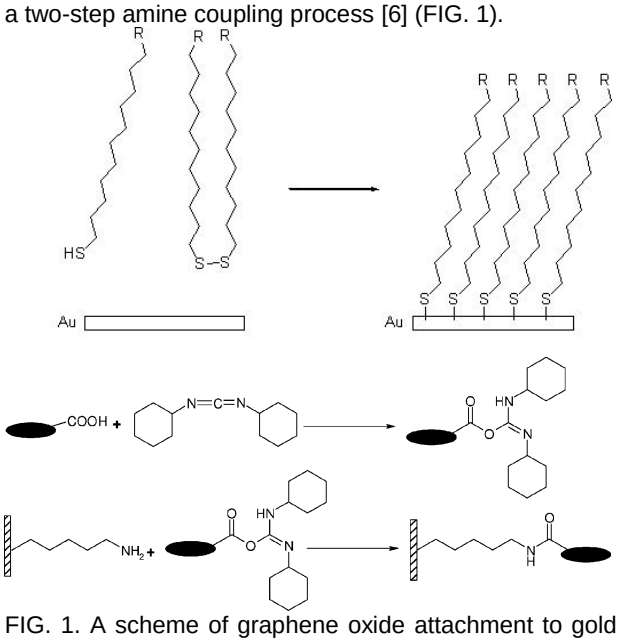

FIG. 1. A scheme of graphene oxide attachment to gold based on creation of SAM on the substrate surface and amine coupling reaction.

Results and Discussion

FTIR spectra confirmed the presence of hydroxyl and carbohydrate moieties on a surface of the modified material (FIG. 2).

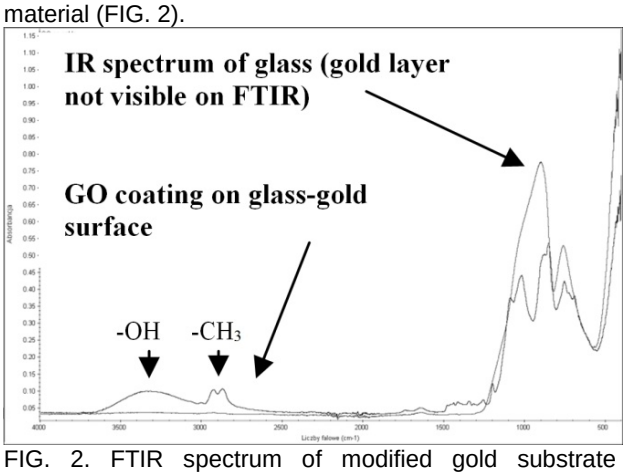

FIG. 2. FTIR spectrum of modified gold substrate compared with bare gold sputtered on glass slide. Moieties from graphene oxide are present.

The modified sensing chip has been used in refractive index measurement experiment and exhibited sensitivity to glucose concentration (FIG. 3).

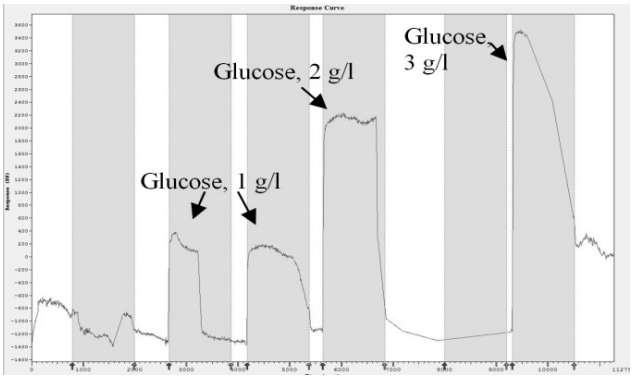

FIG. 3. A response of graphene oxide coated gold refractometric sensor to change in refractive index of the solution (increasing concentration of glucose).

Conclusions

Coatings of graphene oxide onto gold surfaces have been successfully created. It can thus lead to novel biomaterials and biosensor components, however further investigation on biocompatibility and sensing properties needs to be performed.

Acknowledgments

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NEW CEMENT-TYPE MATERIALS BASED ON Ag AND Si DOPED HYDROXYAPATITE FOR BONE REGENERATION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 89]

Introduction

Calcium phosphates (CaPs) are widely used in orthopedic and maxillofacial surgery due to their excellent biological properties. The interesting alternative for high-temperature calcium phosphate ceramics are calcium phosphate cements (CPCs) which are not only biocompatible but also moldable and self-setting. CPCs are valuable and effective bone fillers. Recently they have been also considered as the basis for drug, growth factors or cells delivery systems [1]. The most frequently used CaP is hydroxyapatite (HA, $Ca_{10}(PO_4)_6OH_2$). In recent years more attention has been paid to the ionic substitutions in the crystal lattice of HA. The various ions were introduced into the HA structure in order to improve its physicochemical and biological performance [2]. Simultaneous incorporation of silver and silicon into the structure of HA may lead to development of antibacterial materials with enhanced biological properties. Hydroxyapatite is generally considered to be non-resorbable *in vivo* [3]. In order to improve the resorption rate of HA based materials the addition of more soluble and resorbable phases such us α-tricalcium phosphate (α-TCP), $CaCO_3$ or calcium sulphate (CS) has been proposed.

Materials and Methods

In this work new potential bone substitutes in the form of moldable cement pastes were developed and evaluated. Silver and silicon doped hydroxyapatite (Ag-Si-HA) and α-TCP were synthesized by the wet chemical method. Ag-Si-HA was calcined above 700^{o}C. Three powder batches of the cements were prepared by mixing heat treated Ag-Si-HA with α-tricalcium phosphate, $CaCO_3$ or calcium sulphate. Chitosan or methylcellulose solutions were applied as liquid phases (TABLE 1). The phase composition (XRD, D2 Phaser, Bruker), initial (I) and final (F) setting times (Gillmore Needles), open porosity (Auto Pore IV, Micromeritics) and compressive strength (Instron 3345) were tested. The chemical stability and bioactivity were evaluated *in vitro.*

TABLE 1. Initial composition of the cements.

Material	Powder phase	Liquid phase	L/P [g/g]
A	Ag-Si-HA, CS	chitosan solution	0.68
B	Ag-Si-HA, α-TCP	methylcellulose solution	0.54
C	Ag-Si-HA, α-TCP, $CaCO_3$		0.44

Results and Discussion

Three cement-type materials based on silver and silicon doped hydroxyapatite were obtained. Application of chitosan and methylcellulose solutions as liquid phases improved surgical handiness of the cements. Their setting times differed in the range of 5-13 min (I) and 8-45 min (F). The compressive strength of final cement bodies was from 5 to 8 MPa (FIG. 1).

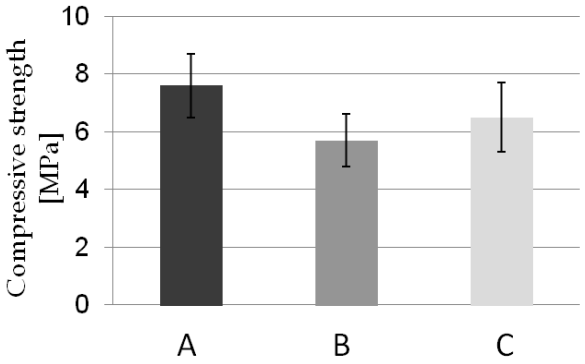

FIG. 1. The compressive strength of cements 7 days after setting and hardening.

Developed materials revealed bimodal pore size distributions with pores below 1.4 μm. Open porosity of the cements was ~50vol.%. Obtained materials showed excellent chemical stability and high bioactivity. SEM observations showed that as soon as after 7 days of incubation in simulated body fluid (SBF) the surfaces of tested materials were covered by the cauliflower-like CaPs structures (FIG. 2).

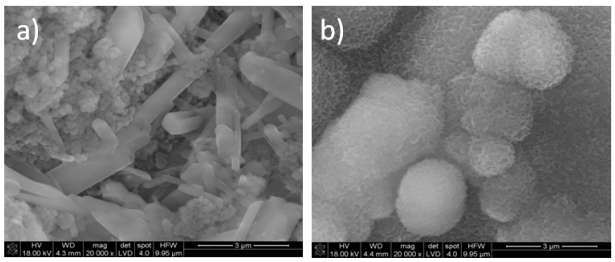

FIG. 2. SEM micrographs of cement A: a) non-incubated and b) incubated for 7 days in SBF.

Conclusions

New bioactive and biodegradable bone substitutes based on Ag-Si-HA and α-tricalcium phosphate, $CaCO_3$ or calcium sulphate were developed. They may be attractive for filling bone defects in the low-load bearing places. Further studies including antibacterial evaluation are conducted.

Acknowledgments

The work has been supported by the Polish National Science Centre funds allocated with the decision DEC-2013/09/N/ST8/04171.

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ELECTROSPINNING AS A FINE TECHNIQUE TO OBTAIN VARIOUS MATERIAL STRUCTURES OF BIO-MEDICAL-PURPOSE POLYURETHANES AND POLYCARBONATES COPOLYMERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 90]

Introduction

Fabrics, films or mixed structures (specific composites) can be produced on electrospinning unit. This multipurpose-technique has been attracted of scientific attention. The less cost of fabrication nano- scale of fibrous materials and electrosprayed film are main advantages of electrospinning. Electrospun fabrics have found wide-spread use in the biomedical field as carriers in drug delivery system, as scaffolds for cell growth purpose, as materials for wound dressing [1-3].

Among the medical-use polymers, the polyurethane (PU) and its copolymers with carbonates and the carbonates copolymers with silicone (PCS) might be performed on electrospinning unit with a well effect. Their attractive mechanical properties and biocompatibility were found place in wide range of biomedical items, such as heart valves, vascular prostheses, and special coatings [4]. Some of these application demand of the multifunctional materials in which one side of the element should be biocompatible and non-adhesive for cells and other side partially degradable with drug release system. These kinds of materials can be obtained on electrospinning unit. Herein, is described the results of preliminary investigations on copolyurethanes non-woven matts obtained via electrospinning and with use more complexed electrospinning-electrospray method.

Materials and Methods

The two types of copolymers were used: Chronoflex Ar 22%(polyurethane-co-carbonate)(PU) and Chronosil AL80A5%(polycarbonate-co-silicone) (PUS) manufa-ctured by AdvanSource. All materials were dissolved in DMAc at concentrations 8% (electrospray purpose) and 18% (electrospinning purpose). The obtained solution was performed at electrospinning unit model NEW-BM (Nabond). The parameters of processing were optimized and finally the best results were obtained for: flow rate of solution, distance between electrodes, the power supply voltage drum collector, needle) humidity. The fibrous materials were obtained using constant parameters during whole process. In contrast the materials formed by combined electrospraying /electrospinning method were obtained with the variable parameters of this process and different weight ratio formed fibres to spray. The morphology of materials was characterized mainly with scanning electron microscopy observation.

Results and Discussion

The conducted experiments revealed that both of copolymers were easily electrospun to the fibrous structures (FIG. 1A). These structures were obtained on various kind of collectors such as solid metal drum, and drum equipped with perforated metal coat (grating). The both of fabrics characterized gradient of fibres compaction which decreased with increasing fabric thickness. The observed phenomena were promising for next experiment in which the PU fabric during electrospinning process were sealed by electrosprayed PCS copolymer. At the first stage of material production, the electrospray of PCS copolymer was mostly performed and the fibres of PU were in minor ratio. At The second stage of process, the PCS electrospray was in the minor compare to PU fibres. The obtained final fibrous sleeve presented two-layer structure, solid in the inner layer and porous outer (FIG. 1B). This results of preliminary investigations show opportunity to form implants of blood vessels, which should present just such a two-layer structure where inner sealed and impermeable, outer porous. The both layers must have a low cells adhesion. The FIG. 1C presents SEM micrograms with zoom of outer layer.

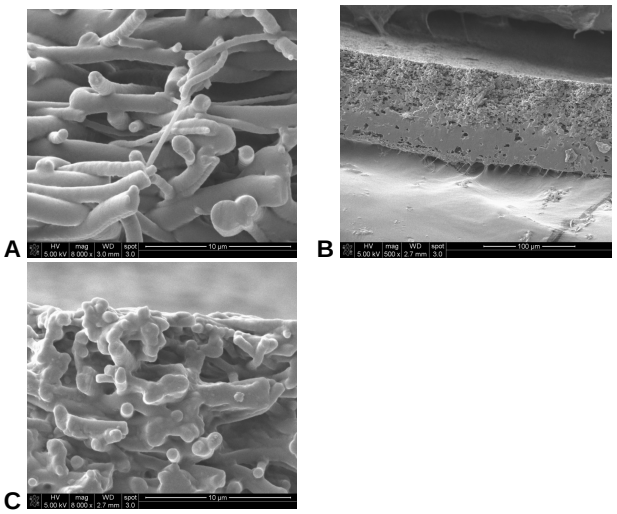

FIG. 1. The SEM micrograms of electrospun and electrosprayed PU and PCS copolymer materials. A) electrospun fabric of PU, B) PU and PCS copolymes materials obtained by mixed method C) the outer porous layer of PU fabric with partially sealed pores formed by electrosprayed PCS.

Conclusions

This previous investigation show opportunity of electrospinning unit and the direction of future experiment. The obtained structure surface will be improved by addition low amount of biodegradable materials fibres. This additive might be a drug delivery system which will work only in possessed direction and controlled time.

Acknowledgments

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CARBON NANOFIBERS FOR MEDICAL APPLICATIONS

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[Engineering of Biomaterials 138 (2016) 91]

Introduction

Carbon nanotubes (CNT) and carbon nanofibers (CNF) are materials with a high potential for medical applications. These carbon forms are interesting candidates for many applications in medical diagnostics and therapies [1,2]. Despite similarities in their form, shape and chemical composition, CNT and CNF display distinctly different surface properties. The differences also result from different structure of both carbon nanoforms.

Materials and Methods

Copolymer with the trade name Mavilon Zoltek Company (Hungary) consisting of 93–94 wt% of acrylonitrile mers, 5–6 wt% of methyl acrylate mers and approximately 1 wt% of sodium alilo-sulfonate mers was used for the manufacture of polymer nanofibers. The polyacrylonitrile (PAN) nanofibers were spun from the polymer solution using the electrospinning method [3]. The transformation of polymer nanofibers into carbon nanofibers consisted of two steps. The first step was the stabilization, during of which the oxidation of the polymer nanofibers took place. The stabilization occurred in an air atmosphere, in two cycles: heating the nanofibers to the temperature of 250^{o}C with the heating rate of 10^{o}C/min, followed by heating the polymer nanofibers to 300^{o}C (heating rate of 10^{o}C) and holding the samples at final oxidation temperature for 20 minutes. The second step was annealing the oxidized samples in nitrogen atmosphere, preventing oxidation of the nanofibers to 1000^{o}C at a heating rate of 5^{o}C/min, without holding the samples at final temperature. The carbonization was carried out in one-step process. The fibers obtained in this manner were then subjected to chemical treatment by the incubation in nitric acid at room temperature. Subsequently, such prepared fibrous samples were treated in the appropriate electrolytes to form on the surface of carbon nanofibers silicon or calcium-contained functional groups to make them bioactive.

Results and Discussion

FIG. 1 and 2 show the FTIR spectra of carbon nanofibers before and after oxidation treatment in nitric acid.

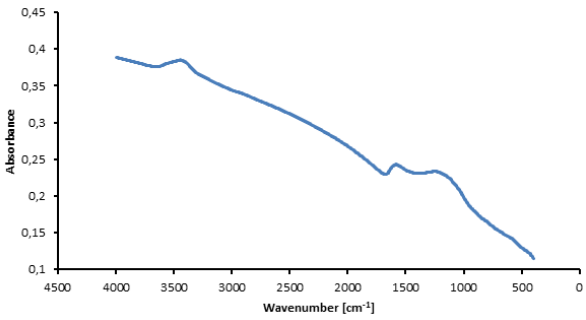

FIG. 1. FTIR spectrum of carbon nanofibers before oxidation treatment in nitric acid (PAN nanofibers after carbonization process).

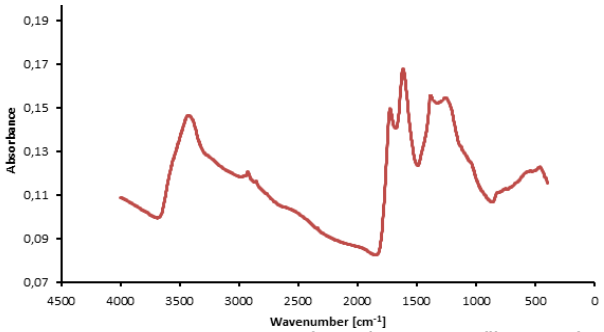

FIG. 2. FTIR spectrum of carbon nanofibers after oxidation treatment in nitric acid.

As seen from the spectra, the incubation process leads to the formation numerous carbon-oxygen-hydrogen functional groups on the fiber surfaces. As it is known such chemical species are desirable for applications in the medical field. Functional groups on the surface of the fiber are crucial in the design of nano fibers- based polymer composites. Oxygen functional groups on the surface of carbon nanofibers enable their further modification by ion exchange and the introduction/ deposition on their surface other elements to create specific biological properties.

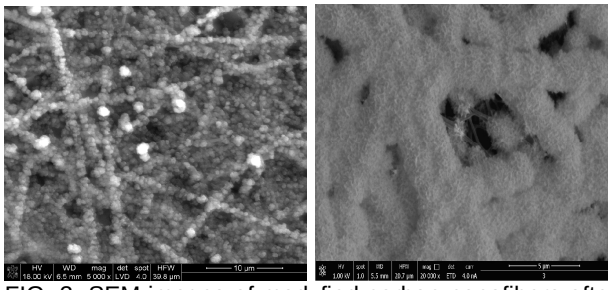

FIG. 3. SEM images of modyfied carbon nanofibers after incubation in simulated body fluid (SBF).

Microscopic images of nanofibers after incubation in SBF indicate that their surfaces are covered with a considerable amount of apatite and have bioactive properties.

Conclusion

Carbon nanofibers, due to their turbostratic structure, are materials which can be effectively modified by means of simple two-step process i.e., oxidation treatment followed by ion exchange process.

Acknowledgments

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NANOCOMPOSITE COATING FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 92]

Introduction

Nanotechnology has opened new possibilities for surface modification of metallic implants designed for orthopedic, dental and maxillofacial surgery [1]. A special role in this domain may play carbon nanotubes. The layers made of carbon nanotubes on a metal surface enable to form a characteristic topography and unique chemical and physical properties of the material providing new opportunities for medical applications, e.g., the hierarchical micro/nanotopography significantly enhances the spreading, adhesion and proliferation of bone cells as well as improves activity of alkaline phosphatase [2-4]. Despite various advantages of such a layer formed by the EPD technique (electrophoretic deposition) on metals, this kind of nonmetallic coating has also some disadvantages resulting from a relatively poor adhesion to the substrate and often unsatisfactory interactions between the nanotubes forming layer. The aim of the work was to manufacture a nanocomposite coating on a metal surface, containing carbon nanotubes, free from drawbacks that are associated with the carbon nanotube layers obtained in the EPD process.

Materials and Methods

The study was based on carbon nanotubes subjected to the process of functionalization and a sol prepared from alkoxysilanes (dimetylodietoksysilanu and methyl triethoxysilane molar ratio of 2: 1), HCl catalyst and ethanol as solvent. Layer on the titanium surface, was prepared in a two-step method; EPD + sol-gel. In the first the appropriately prepared substrate was coated by a layer of carbon nanotubes which are then coated with sol and subjected to drying. The objectives of this study were the nanocomposite layer CNT/SIL and a layer made of carbon nanotubes. The two types of layers were subjected to microscopic examination. Then assesses the adhesion of layers to the substrate using the scratch test. Both materials were incubated in a physiological fluid (SBF) to determine their bioactivity. Cytotoxicity of these materials was determined in contact with the MG-63 cells, using the LDH assay which detected level of lactate dehydrogenase release from cells growing in the presence of materials. Also we conducted studies of genotoxicity of materials based on test γ-H2AX as a biomarker of DNA damage - double-strand breaks – dsb.

Results and Discussion

The results of microscopic nano-composite layer are shown in FIG. 1. From microscopic study indicate that nanocomposite layer CNT/SIL retains the topography characteristic of the layers produced from the carbon nanotubes. The sol gel process that takes place in the presence of carbon nanotubes leads to a solid layer, built of carbon nanotubes with surface modified by silanol groups. From the study of scratch testing indicate that the destruction of the CNT layer is starting already at a force of approx. 1 N and that in the case of a nanocomposite layer is followed at a fraction of the higher strength of about 45 N. The total destruction layer formed nanocomposite will occur at a much higher strength as compared with the layer made of the same CNT.

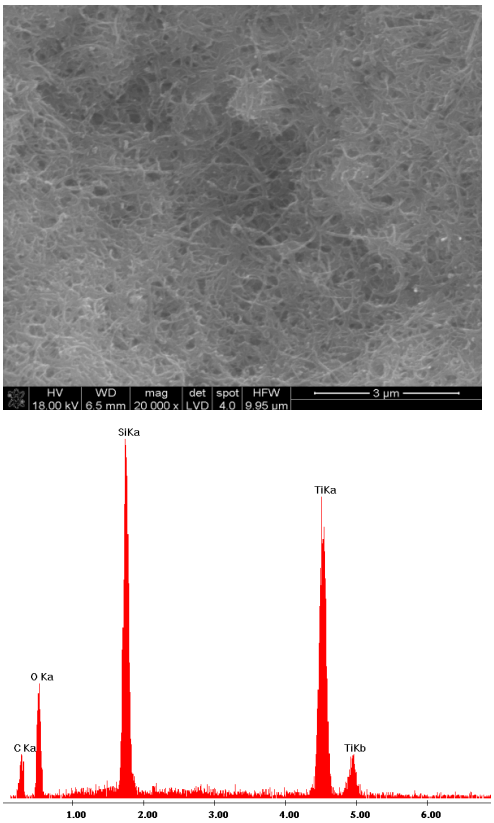

FIG. 1. SEM image and elemental analysis of CNT/SIL layer on titanium surface.

The results of the cytotoxicity study of titanium and both types of coatings are shown in TABLE 1.

TABLE 1. LDH release from MG63 cells in contact with Ti surface and CNT, CNT/SIL coating on metal surface.

Time of culture	PS (control)	Titanium surface	CNT coating	CNT/SIL Coating
1 day	6.3±0.3	11.0 ±1.3	7.4±0.9	8.2±0.6
3 days	7.8±0.3	11.6±0.7	8.0±0.2	6.1±0.5

The results of LDH test performed after 1 and 3 days of culture are significantly better for both types of layers in the entrainment of the titanium surface (TABLE 1). Produced, under the working, nanocomposite layer CNT/ SIL are non-cytotoxic and non- genotoxic as well as have a high bioactivity in contact with the artificial plasma.

Conclusions

Two-stage method for the modification of metal implants leads to the production of layers which use a high potential of carbon nanotubes to modify biomedical devices also can provide a material without the disadvantages that may accompany the processes for producing coatings of carbon nanotubes in a EPD method.

Acknowledgments

This work has been supported by the NCN-National Science Center, Poland) grant: UMO-2014/13B/ST8/01195.

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HYALURONIC ELECTROSPUN MEMBRANES AS ACTIVE SCAFFOLDS FOR BONE AND CARTILAGE TISSUE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 93]

Introduction

Biomimetic fibrous structures are the subject of scientific interest due to their presence in many human tissues. Thanks to the electrospinning method it is possible to obtain fibers with a submicrometric and nanometric diameter. Hyaluronic acid (HA) is a biopolymer present in many tissues in a fibrous form. It is one of the main components of the extracellular matrix (ECM), contributing to the growth of bone, cartilage and skin tissues [1]. Its physicochemical properties assure high absorbability, thus HA supports the barrier function against outside factors and it contributes to tissue hydrodynamics (firming effect) [2-3]. According to the literature, the morphology of the fibrous substrate depends on the forming conditions (i.e. on solvents) [3]. Considering application of the material as a biomimetic substrate, the best diameter of fibers is 50-250 nm. Unfortunately, in many cases the literature does not present the data concerning biocompatibility of the substrate, whereas bioactivity is a key factor in medical applications. The aim of this work is to select conditions for biological tests on fibrous substrates. Biochemical monitoring was conducted to assess cytotoxicity of the materials. The L929 cells growing in contact with the extracts of the materials underwent the tests of cytotoxicity and proliferation.

Materials and Methods

Commercially-available biopolymer Centiprio HA of molecular weight 1.8-2.0kDa was used in the study. Three types of HA fibers were obtained by means of electrospinning and then examined: pure HA fibers - 12% HA 2:1 WAM:DMF (Avantor), fibers with medication (abf) - 12% HA 2:1 WAM:DMF+3% Biofuroksym (Polpharma) and fibers with HAp - 11% HA 2:1 WAM:DMF+1% HAp (Sigma Aldrich). The morphology of fibrous materials was assessed by means of SEM (Nova NanoSEM). The presence of additives (abf, HAp) was established during EDS analysis (Genesis). Biological tests were conducted on fibroblast cell line L929, cultured in EMEM medium with 10% of fetal bovine serum at 5% CO_2 /37^{o}C). The reference was the surface of cell culture plate wells (TCPS). TCPS was a negative control for cytotoxicity tests. Cell tests were run on 3. and 7. day of cell cultures in the presence of extracts of the tested materials. The primary biochemical analysis was cytotoxicity assay (ToxiLight, Lonza) and the test of total number of cells in the culture (ToxiLight 100% Lysis Control, Lonza). For each of the conducted tests the statistical analysis was run as well (t-student test, $p < 0.05$).

Results and Discussion

Both the biopolymer pure HA fibers and the modified ones (HA/HAp, HA/abf) display a similar range of diameter: 50-150nm. Extracts formed above all the materials (HA, HA/Hap, HA/abf) amounting to 1 ml of the tested material/1ml medium (called 0.5) or 0.2ml/1ml medium (called 0.2) showed cytotoxic effect on cell cultures. Hyaluronan expands in hydro-environment (it is a hydrogel) and at higher concentrations it prevents free gas exchange. Introducing an adequate amount of hyaluronan *in vivo* will probably result in colonisation of the outer layer of biomaterial and next metabolizing HA by surrounding cells. In the case of extracts of lower concentrations (i.e. 0.1ml/1ml medium, 0.05ml/1ml medium and 0.025/1ml medium) the cytotoxicity is disappearing gradually and there are smaller differences between the materials modified with hydroxyapatite or antibiotics (FIG. 1). For all the tested materials at concentrations of 0.1, 0.05, 0.025, it is a rule that the cell count at least doubles in a 7-day culture as compared to the 3-day one (FIG. 2), while relative cytotoxicity halves. It proves advantageous conditions of cell cultures that support adaptation and proliferation of cells.

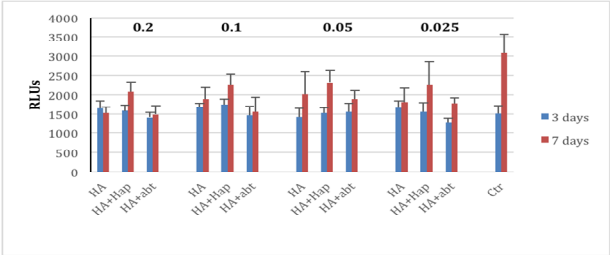

FIG. 1. Cytotoxicity of consequtive concentrations of studied materials after 3 and 7 days in *in vitro* cell culture conditions. RLUs – relative luminescence units.

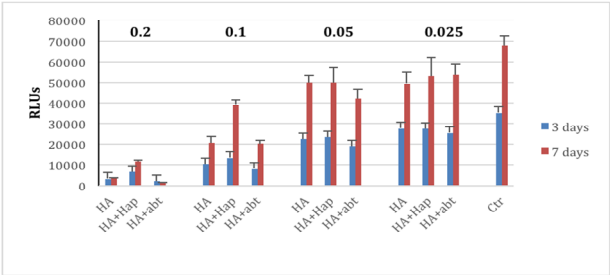

FIG. 2. Relative number of fibroblast cells after 3 and 7 days of *in vitro* culture with examined materials. RLUs – relative luminescence units.

Conclusions

Effectiveness of electrospinning as a method to obtain fibers depends on the ratio of solvents (WAM:DMF). Biological tests conducted at low concentrations of HA - considering its hydrogel characteristics - give credible results. The material itself is biocompatible: it does not cause cytotoxicity and it facilitates adaptation and proliferation of cells. However, it is necessary to carry out further research to assess how the materials behave in prolonged contact with other cell lines e.g. chondrocytes and osteoblasts.

Acknowledgments

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CONTROL OF THE BIOLOGICAL RESPONSE TO METALLIC BIOMATERIALS THROUGH APPLICATION OF THE DLC COATINGS WITH DOPANTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 94]

Introduction
Increasing market of implants and medical devices enhances nowadays the diversification of products that support surgical outcome more successfully [1]. Currently, two groups of materials the most of implants are made of, are metals and polymers. The commonly applied metallic implants possess relatively poor surface properties caused by the lack of total chemical stability in human body environment [2]. One of the most extensively researched solutions include the application of diamond-like carbon (DLC) coatings, which exhibit a combination of highly desirable properties in the context of biomedical applications [3]. Different properties of synthetized carbon coatings can be further improved by the incorporation of different elements into the carbon matrix [4]. As far as orthopaedic and cardiovascular implants are concerned, one of the most promising dopants is silicon (Si), which not only favours the proliferation of endothelial cells, but also acts as an antithrombogenic agent. In the case of orthopaedic implants the enhancement of the osseointegration process is highly desirable in order to assure the proper bone-healing, what may be achieved by the addition of either titanium (Ti) or silicon. In the case of implantation procedure, in general, the antibacterial properties are very important. Addition of silver can fulfil this requirement [5].

Materials and Methods
In this work the DLC coatings with Si and Ag dopants were have been manufactured on two commonly applied metallic biomaterials (AISI 316 LVM steel and Ti6Al7Nb alloy) using two methods: RF PACVD and magnetron sputtering. The surface characteristics involved the analysis of surface morphology (SEM), chemical composition and structure (XPS, FTIR) as well as surface wettability and surface free energy. The biological assessment of the deposited coatings was based on two complementary cell proliferation and viability assays (LIVE/DEAD and XTT test) performed on two different cell lines, i.e. endothelial cells line EA.hy926 and osteoblast-like cells line Saos-2. The bactericidal activity was assessed using E. coli. The obtained results allowed to check the influence of both dopants on the biological response towards the modified carbon coatings as well as to correlate the obtained results with the surface properties of the investigated coatings.

Results and Discussion
According to the literature, the increasing concentration of Si is associated with lower number of adhering platelets and decreased platelet activation level, and hence higher hoemocompatibility [6]. At the same time, Si favours the attachment of human endothelial cells and does not induce cytotoxicity [7]. Our investigations showed that dopant has to be incorporated at proper rate to induce positive reaction in biological meaning constituting a safe implant with designed properties. Too high amount of Si does not improve the hoemocompatibility and depending on the methods and substrate, the rate of Si as well as Ag element has to be individually adjusted.

Conclusions
The obtained results demonstrated that the incorporation of Ag and Si dopants (at proper ratio) allows to obtain coatings of good biocompatibility and high antibacterial properties, what makes them a good materials for medical application.

Acknowledgments
This research has been supported by the National Centre for Research and Development under the grant no. LIDER/040/707/L-4/12/NCBR/2013 entitled „MOBIOMED: Modified BIOmaterials – MEDicine future" and grant no. M-ERA.NET/2012/02/2014 entitled "CarLa – Ag/Si doped carbon layer for bio-medical application".

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XRD AND MORPHOLOGY ANALYZES OF HIGH-NITROGEN Fe-Cr-Mn ALLOY POWDERS SYNTHESIZED BY MECHANICAL ALLOYING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 95]

Introduction

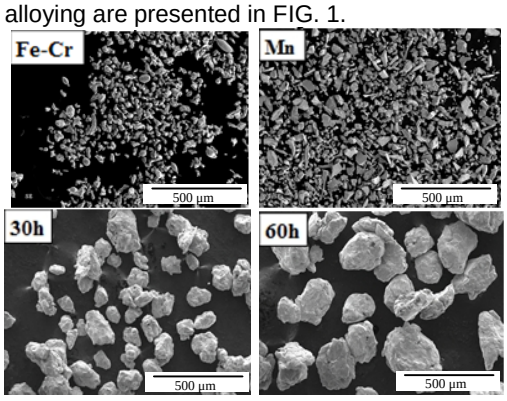
Nowadays, nickel-free high-nitrogen austenitic stainless steels are attractive materials in the biomedical field. They can be substitutes for Cr-Ni stainless steels, due to biocompatibility, good corrosion resistance and mechanical properties [1-3]. Nickel is an element occurring in conventional steels. It's a toxic element which causes allergies [4]. Nitrogen can be a replacement for nickel. The addition of N not only stabilizes the austenite phase but also causes beneficial effects on the steel properties, such as better corrosion resistance and improved mechanical properties and [3,4].

Materials and Methods

A prealloyed Fe-Cr powder were mechanically alloyed (MA) with 18%Mn (in wt.%) powder with powder size 45µm and purity of 99,95%. MA process was performed in a high-energy ball mill Pulverisette 5 (Fritsch, Germany) equipped with a stainless steel jar and balls with a rotation speed of 250 rpm, in pure nitrogen atmosphere (99.998%). Ball to powder weight ratio of 10:1 was used. At selected times a small amount of as-milled powder was taken out for further morphology and XRD analyses. To minimize air contamination of the powder, loading and unloading of the powder was performed in an argon glove box. MA process was conducted up to 150 h until the solute elements peaks in XRD patterns had disappeared. The crystallite size was determined by measuring the Bragg peak width at half the maximum intensity using Sherrer formula [5].
The powder morphology was studied using scanning electron microscopy (SEM), equipped with an energy dispersive spectrometry (EDS) system.

Results and Discussion

The morphological changes exhibited during mechanical alloying are presented in FIG. 1.



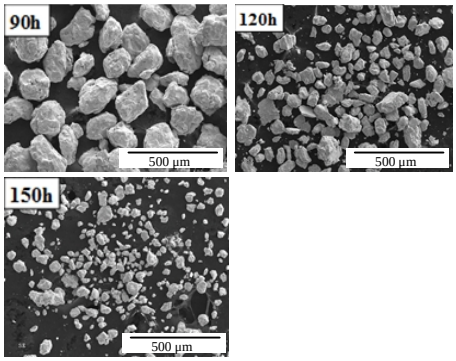


FIG. 1. SEM images of as-milled Fe18Cr18Mn powders at different processing times: 30, 60, 90, 120 and 150h.

The particles of unmilled Fe-Cr powder are irregular in shape and particles of Mn powder are flattened. At the early stage of the process (30h) the particles are deformed, but after 90h particles are more oval in shape. After 120 hours, two types of particles are observed, the small ones are more regular in shape, while the bigger ones are deformed. The particle size increases until 60 hours of a milling time and then decreases. After 150 hours size of a single particle reaches 9.86 µm. Cisneros M.M. et al. [4] shows similar researches, but he presents various shapes of powders at different times of milling.

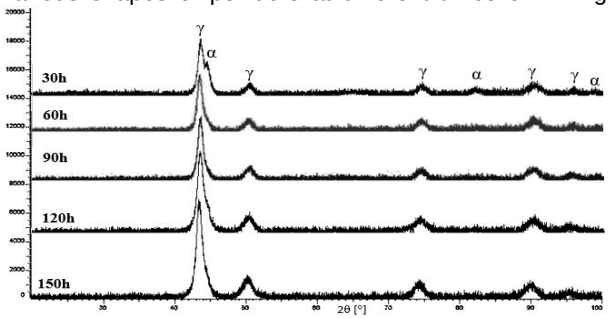


FIG. 3. XRD pattern of Fe-18Cr-18Mn powder milled.

After 30 hours of MA under argon atmosphere, at the first peak the austenitic-ferritic phase occurs, but after 60 hours, when the atmosphere is changed into nitrogen, the ferritic structure disappears and the dominant crystalline phase is austenitic (FIG. 3). With progression of the MA process the intensity of the XRD peaks is increased. Amini R. et al. [3] shows that after 24 hours of MA in nitrogen atmosphere the dominant phase at the first peak is ferrite. However, in his research, powders with less amount of manganese were analysed (8% Mn and 18% Cr). After 60 h the crystallite size was 10.30nm, then decreases and after 150 hours of MA it reached 8.38nm.

Conclusions

The nickel-free austenitic stainless steel Fe18Cr18Mn was produced by mechanical alloying under argon atmosphere for the first 30h and then under nitrogen atmosphere. In the beginning of researches the particle size was 45µm, and after 150 hours milling it reached 9.86µm. The material structure was transformed from an α-phase into γ-phase. The intensity of XRD peaks increased along with duration of the MA.

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HYDROLYTIC DEGRADATION OF PLLA AND PLGA WITH SURFACE MODIFIED BY CO_2 LASER – PRELIMINARY STUDY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 96]

Introduction

Biodegradation of the polymeric medical devices is relevant from the application point of view. For some function not only the adequate shape plays important role but also behaviour of the device during the implementation time. In order to adjust degradation in specific areas surface modification of materials are performed [1-4].
In order to determinate influence of the laser irradiation on the hydrolytic degradation of polymer 3 group of materials, $PLLA_{AMO}$, $PLLA_{CRY}$, PLGA with surface irradiated with CO_2 laser of different powers, were incubated for 5 weeks. The aim of the study was to investigate the differences in mechanical properties of the biopolymers after degradation time in relation to the analysed material and used laser power.

Materials and Methods

The polymer sheets having an average thickness of 300-400 μm were extruded from commercial medical poly(L-lactide) (PLLA Evonik L210S) and poly (L-lactide-co-glycolide) (PLGA, Evonik LG857s) by compression molding of the granules pre-heated up to 200°C. In order to prevent material adhesion to the mold half polyamide spacer were used (Kapton HPP-ST, thickness 127 μm). This procedure allowed to obtained amorphous poly(L-lactide) ($PLLA_{AMO}$) sheds having the degree of crystallinity Xc ≈ 2%. The crystalline polymer ($PLLA_{CRY}$) was obtained from amorphous specimens which underwent thermal crystallization process for 5h in 100°C.
In order to investigate the influence of the CO_2 laser surface modification on the hydrolytic degradation of the polymer specimens were irritated with two laser powers P_1=24mJ/cm^2 and P_2=71mJ/cm^2. All samples were placed in demineralized water and incubated in 37°C for 5 weeks. Finally the mechanical properties of the specimens were determined in tensile test [5]. The stress-strain curves were determined and on the basis of obtained curves the tensile strength R_m and Young's modulus were calculated.

Results and Discussion

Presented preliminary study intend to determinate the behaviour of the most common biopolymers irradiated with CO_2 laser under hydrolytic degradation in relation to mechanical properties. From analysed materials laser beam had the strongest impact on PLGA for which decrease in tensile strength R_m average of 20% and 80% respectively for laser power 24 mJ/cm^2 and 71 mJ/cm^2 was observed. Moreover Young's modulus E decreases up to 45% with higher laser power.
In the case of PLLA, depending on its crystallization form CO_2 laser causes deferent effects on polymer. For amorphous poly(L-lactide) with the increase of laser power the decrease of 10-45% in tensile strength R_m and decrease of 5-37% in Young's module were observed.

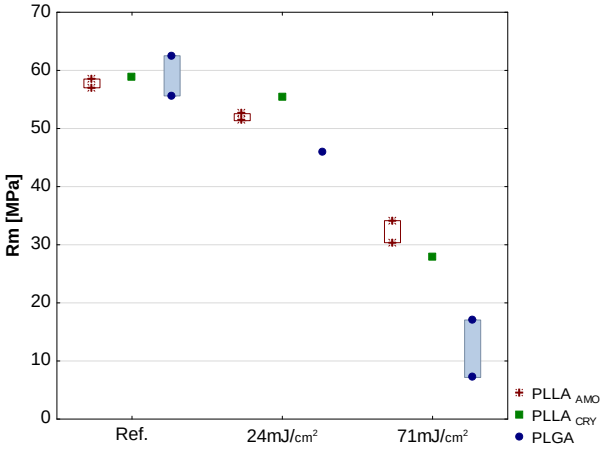

FIG. 1. The tensile strength R_m [MPa] of the biodegradable polymers, $PLLA_{AMO}$, $PLLA_{CRY}$ and PLGA irradiated by CO_2 laser of two powers.

The crystalline form of PLLA shown dual response on CO_2 laser irradiation. For power 24 mJ/cm^2 the 5% decrease of R_m with 18% increase of Young's was noticed. However for power 71 mJ/cm^2 tensile strength reduce to half while Young's modulus is similar to reference material $PLLA_{CRY}$.

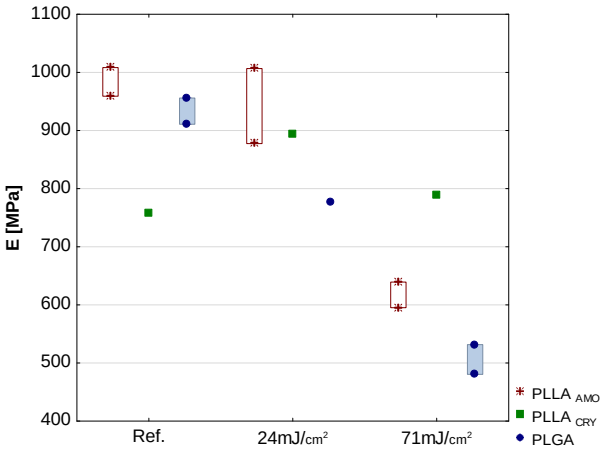

FIG. 2. The Young's modulus E [MPa] of the biodegradable polymers, $PLLA_{AMO}$, $PLLA_{CRY}$ and PLGA irradiated by CO_2 laser of two powers.

Conclusions

Conducted investigation aimed to show the changes in hydrolytic degradation of various material modified by the CO_2 laser. Study reviled the relationship between the degradation rate and the increasing power of the laser. Moreover laser beam has stronger influence on the PLGA than PLLA, regardless the crystallisation form, what can be noticed in grater decrease of mechanical properties. Preliminary study were crucial in order to determinate the rate of degradation and plan efficient long-term experiment.

Acknowledgments

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THE INFLUENCE OF POLY(L-LACTIDE) SURFACE MODIFICATION BY CO_2 AND EXCIMER LASERS ON MECHANICAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 97]

Introduction

Laser surface modification of the polymers can significant influence on the physicochemical and mechanical properties. Depend on the used laser as well process parameters e.g. the length of the light wave, pulse duration, number of pulses or pulse energies obtained results may be different [1-4].

The aim of the study was to investigate the influence of the poly(L-lactide) surface laser modification carried out by two types of laser: CO_2 and excimer ArF on its mechanical properties. According to the best of authors knowledge there is limited number of publication describing the influence of laser irritation on the mechanical properties of the biodegradable polymers for medical application.

In order to determinate the mechanical properties of PLLA specimens irritated by lasers underwent tensile test where form obtained stress-strain curves the tensile strength R_m and Young's modulus E were calculated.

Materials and Methods

The polymer sheets having an average thickness of 300 – 400 μm were extruded from commercial medical poly(L-lactide) (PLLA Evonik L210S) by compression molding of the granules pre-heated up to 200°C. In order to prevent material adhesion to the mold half polyamide spacer were used (Kapton HPP-ST, thickness 127 μm).

This procedure allowed to obtained amorphous poly(L-lactide) sheds having the degree of crystallinity Xc ≈ 2%.

In conducted study two types of laser were used: CO_2 laser with wavelength λ=10,6 μm and maximum average power of 25W. For surface modification three powers were used P_1=24mJ/cm^2, P_2=48mJ/cm^2 and P_3=71mJ/cm^2 (i); excimer laser ArF where PLLA samples were irradiated with respectively 20, 40 and 80 impulses per unit of area with same fluence (ii).

The choice of the applied CO_2 laser powers and number of excimer laser impulses were aimed to obtain the same geometrical outcome on the specimens' surface. For better comparison, in FIG. 1 and FIG. 2, comparative laser modification effects were separated by a dashed line.

Results and Discussion

Based on the obtained stress-strain curves the tensile strength R_m and Young's modulus E were calculated. The parameters describing the mechanical properties of the modified PLLA specimen are presented in FIG. 1 and FIG. 2. Conducted studies shown that surface modification of PLLA by CO_2 laser results in gradual decrease of the tensile strength R_m and increase of the Young modulus E in respect to the reference group. The slightest changes were observed for CO_2 laser for power 24mJ/cm^2 where R_m decreased 3.4% while Young modulus increased 0.8%. With the increase of the laser power up to 48mJ/cm^2 causes decrease mechanical properties of 14.5% and 1.1% for tensile strength and Young modulus respectively. The most destructive impact on PLLA had CO_2 irradiation of power 71mJ/cm^2 where the R_m value is nearly half of the one obtained for reference group while Young's modulus E raise up to 6%.

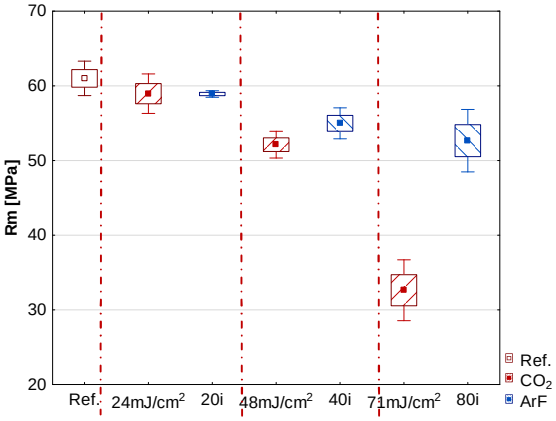

FIG. 1. The tensile strength R_m [MPa] of the reference PLLA and modified by CO_2 and excimer ArF laser.

Changes in mechanical properties of the samples modified by excimer ArF laser are not as significant as for the CO_2 laser. The tensile strength R_m decreases from 3.4%, 9.9% to 13.7% respectively for the 20, 40 and 80 impulses. Irradiation of 20 impulses per area results in increase of the Young modulus up to 2.6% and consequently increasing number of impulses applied on the PLLA surface will lead to a further reduction of the E respectively 1.3% and 3.3%.

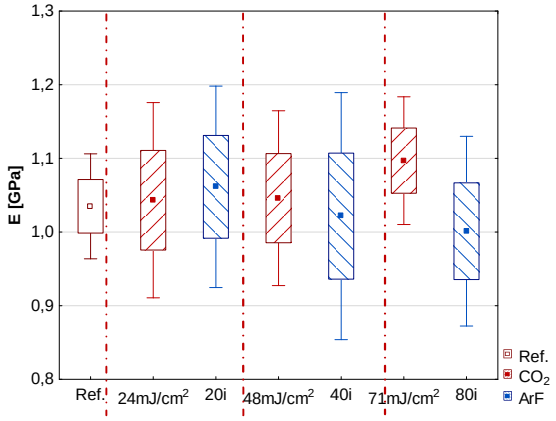

FIG. 2. The Young's modulus E [GPa] of the reference PLLA and modified by CO_2 and excimer ArF laser.

Conclusions

Conducted studies shown that the CO_2 laser irradiation has stronger impact on PLLA than the excimer ArF laser while maintaining geometrical modification of specimens.

Acknowledgments

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ELECTROPOLYMERIZED POLYMER COATINGS FOR STAINLESS STEEL

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[ENGINEERING OF BIOMATERIALS 138 (2016) 98]

Introduction

Stainless steel (SS) 316L is often used for medical applications, e.g. for fabrication of stents or artificial valves. However, these life-saving devices have negative long-term influence on tissues of a patient. Adsorption of platelets and leukocytes leads to formation of blood clots and inflammation [1]. Also, steel releases toxic ions (e.g. Cr or Ni); in the case of stents there is also a problem of restenosis [2]. Many approaches has been undertaken to solve these problems [3], however none of them seems to be good enough. In present paper we claim that it is possible to obtain biocompatible and hemocompatible polymer coating for steel that could also be functionalized and grafted with endothelium-specific peptides.

Materials and Methods

Polymer coating on SS 316L flat discs (ø14mm) was obtained through electropolymerization method. SS disc was dipped in the aqueous solution containing poly(glycol dimethacrylate (PEGDMA), crosslinking/polymerization substrate - ammonium persulphate ($(NH_4)_2S_2O_8$), crosslinking agent - ethylene glycol (EGDMA) and additives improving the quality of coatings. DC power supply was used as a power source. Decomposition of $(NH_4)_2S_2O_8$ triggered by passage of current led to radical crosslinking of PEGDMA resulting with forming a crosslinked PEGDMA (cPEGDMA) coating on SS surface. cPEGDMA coating was functionalized thanks to addition of acrylic acid (AA) to the reaction solution. Then peptide molecules containing adhesive sequence (GSGREDVGSG) were grafted to superficial COOH groups utilizing EDC/sulfoNHS protocol. cPEGDMA coating was analyzed with the use if SEM and FTIR-ATR. The concentration of AA and time of AA electropolymerization was tested using TBO method of COOH groups quantity. Peptide presence was determined e.g. via BCA colorimetric assay.

Results and Discussion

FIG. 1 shows FTIR-ATR spectra of SS surface coated with AA at AA concentration of 1%(v/v) and different reaction times: 5, 15 and 30min. Characteristic peaks indicating chemical groups are highlighted. The intensity of the peak at 3400cm^{-1} is the smallest for SS-PEGDMA (at the bottom) and the biggest for 30min of reaction (at the top). So, as reaction time increases, the number of COOH is increasing. It is understandable since the longer time of AA polymerization, the more monomers are incorporated in the polymer chain, thus the more COOH groups are present. It was also reported by other authors who used the same method of COOH groups quantification in the study considering polyurethane scaffolds radically grafted with AA [4].The effect of AA concentration was also studied. It was demonstrated that for higher AA concentrations the COOH content is increased, which corresponds to other works [4,5].

FIG. 2 presents BCA assay results for carboxylated SS chemically grafted with adhesive peptide (marked as SS-REDV edc/nhs, two sets of carboxylation parameters were used: 30% (v/v) of AA, 30min and 1% (v/v) of AA, 5min) with comparison to SS alone (SS) and carboxylated SS incubated, not grafted with peptide solution (marked as SS-REDV ads) in order to see how much physical adsorption of REDV alters the results. SS-REDV edc/nhs seems to pisses the biggest number of REDV molecules, however it is also significantly adsorbed in polymer matrix. Since SS also gives a high response in BCA assay, another method of REDV evaluation is needed.

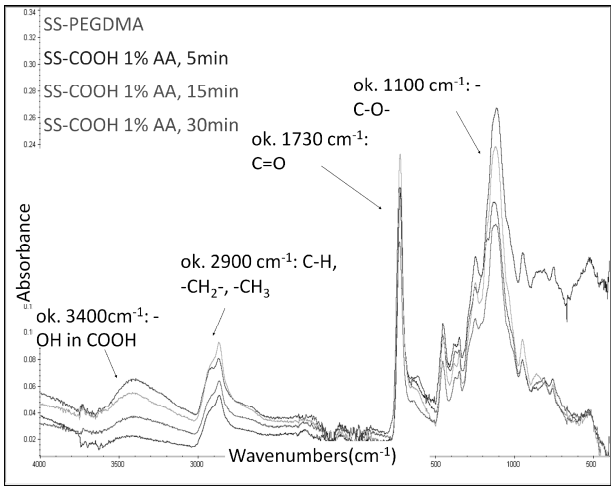

FIG. 1. FTIR-ATR spectra of SS surface coated with AA at AA concentration of 1%(v/v) and different reaction times.

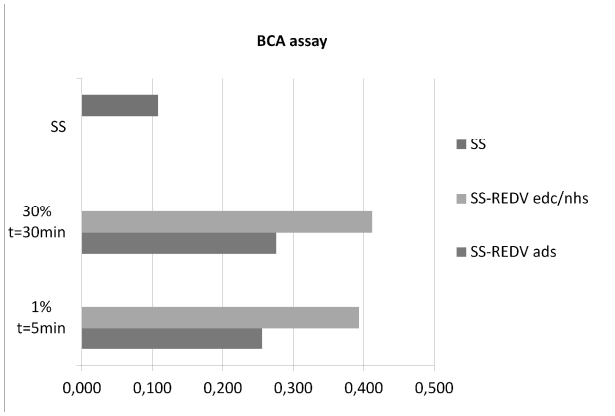

FIG. 2. BCA assay results for carboxylated SS chemically grafted with REDV peptide (SS-REDV ads), carboxylated SS with REDV adsorbed, not grafted and SS alone. Two sets of carboxylation parameters were used.

Conclusions

It has been demonstrated that SS can be coated with polymer layer that offers a potential of functionalization. Superficial carboxyl groups could be used for peptides grafting. More results will be shown including more detailed polymer coating characteristic as well as endothelial cells culture of SS-REDV surfaces.

Acknowledgments

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CHARACTERISATION OF THE BONE CEMENTS ON THE BASIS OF PMMA AND ORGANOSILICON POLYMERS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 99]

Introduction
Bone cements are widely used in vertebroplasty, kyphoplasty, filling the bone ullage and in stabilization of fractures. The endprosthesis' fastening is also their application. Bone cements should meet the following requirements: have the short polymerization shrinkage, own relatively big hardness, do not emit huge amount of heat during polymerization's process, should be easily injected-have moderate viscosity, be bioactive and biocompatible and have short time of cohesion. There are a few groups of bone cements: polymeric, calcium-phosphate, composite, hydrogel one [1].
The aim of the study was an examination of physico-chemical and biological properties of the silicon and polymethyl metacrylate bone cements and their comparison.

Materials and Methods
During research the commercial bone cements were used: three on the basis of polymethyl metacrylate (PMMA) with different excipients and the silicon one. The analysis of cements' morphology and the map of location the respective compounds in polymieric matrix was conducted using SEM microscope (*Scanning Electron Microscopy*). The chemical structure was examined using FTIR spectroscope (*Fourier Transform Infrared Spectroscopy*). Technique DSC (*Differential Scanning Microscopy*) enabled to establish the amount of heat released during polymerisation. Besides, the time of cements' hardening was measured using direct measurements of hardened mass' temperature and the hardness' measurements in the Shore's scale in two different temperatures. The wettability of cements' surface was established based on the geometrical structure of water drop located on the examined surface. SEM was used in order to estimate biological bone cements' properties -the blood plates' level of activation and aggregation.

Results and Discussion
The morphology measurements demonstrated that manual mixing of powder and liquid enables the regualr relocation all of their ingredients in polymeric matrix. The differences in surface's morphology and topography are the result of usage different excipients. FTIR measurements ratified the presence of all chemical structures which came from organic and non- organic compounds of the examined cements.
The results of DSC showed that in temperature 27 and 37ºC the bone cements on the basis of PMMA emit about 85 J/kg heat and silicon cements emit only 3.7 J/kg heat. The essential result is also the time of polymerisation – for the bone cements on the basis of polymethyl metacrylate it took respectively 7 min and for the silicon one – 50 min. In temperature 37ºC, the time of polymerisation is definitely shorter – 3 min 18 s. Additionally, the time of polymerisation was ratified by the hardness' measurements. 90 HSA was established as a "final" hardness for cements consist of PMMA and 10 HSA – for silicon bone cements. The direct measurements of temperature confirmed that maximal temperature for hardened PMMA cements was 82ºC, and for the second group of bone cements, it was 30ºC. The surfaces of each examined cements were hydrophobic. The biological researches of the bone cements enabled the quantitative and qualitative estimation of the blood plates' activation and aggregation on their surfaces.

Conclusions
On the grounds of conducted researches the eveness of respective compounds in polymieric matrix was established. Used excipients have huge impact on cements' morphological structure. In chemical structure of cements the non-polar bonds dominate, what influences on surface's hydrophobicity. Examined biomaterial characterised with good contact with blood. All of presented results are connected with work on the mark the most suitable material for innovative surgical technique used to treat degenerated spine.

Acknowledgments
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ATHROMBOGENIC DIFFUSIE TiN SURFACE LAYERS APLICATION AND EVALUATION IN POLISH IMPLANTABLE ROTARY CONTINOUS FLOW BLOOD PUMP

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[ENGINEERING OF BIOMATERIALS 138 (2016) 100]

Introduction

The implantable rotary continuous flow blood pump, ReligaHeart ROT has been developed [1,2], as an implantable left ventricular assist device for patients with advanced heart failure.
The surface engineering was used to create blood pump elements surfaces structure, in order to reduce shear stress induced platelet activation on blood contacting pump elements and avoid blood clothing inside the pump. The innovative process of plasma glow discharge technology and detail appropriate surface structure and topography has been developed and confirmed first in laboratory testing [3]. The titanium nitride (TiN) surface layers proper haemolytic properties as well as athrombogenic properties (platelets activation and adhesion after contact with blood) were confirmed in vitro, under high shear stress conditions [4].
The next step of biomaterial evaluation was TiN layers application in ReligaHeart ROT device and examination of its surface structure, topography and selected biological properties (biocompatibility with blood).

Materials and Methods

The ReligaHeart ROT prototypes, with magnetically and hydro-dynamically suspended impeller, producing flow up to 10 l/min were manufactured. The athrombogenic diffusive titanium nitride TiN+Ti_2N+αTi(N) layers have been applied for titanium pump's house and rotor, with roughness of Ra=80nm. The TiN surface zone was examined using TEM, SEM and AFM.
The 6 hours acute thrombogenicity test [5] on fresh animal blood was performed to validate the ReligaHeart ROT pump embolization risk (FIG. 1).

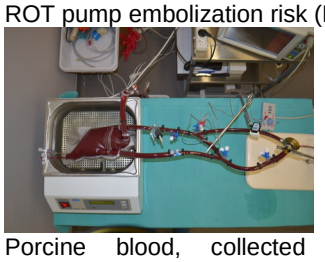

FIG. 1. The acute thrombogenicity tests.

Porcine blood, collected in slaughterhouse and anticoagulated by means of heparin in dosage of 1,5u/ml, was used. This qualitative test shows if the device made of investigated biomaterial tends to form and adhere thrombus under continuous blood flow conditions. The ReligaHeart ROT prototypes (n = 3) were examined. The circulation time was: 220, 260 and 280 min (up to reach critical ACT = 1,5*animal's ACT). The circulation parameters were: blood flow = 5lpm, afterload pressure = 100mmHg. ACT and platelet number in time during the experiment was measured. After the test the pump were disassembled and detail assessment of pump elements were performed in order to evaluate the potential thromboembolic material collected inside the pump.

Results and Discussion

The nitride layers with nanocrystalline titanium nitride (TiN) surface zone TEM, SEM and AFM examination confirmed the appropriate structure and topography on the whole blood pump elements (FIG. 2, 3).

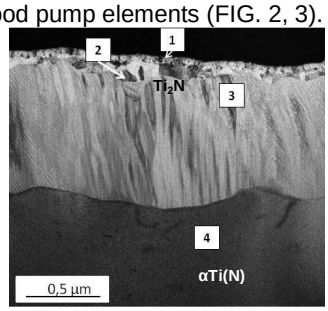

FIG. 2. Surface structure of TiN+Ti_2N+αTi(N) layers on the blood pump elements.

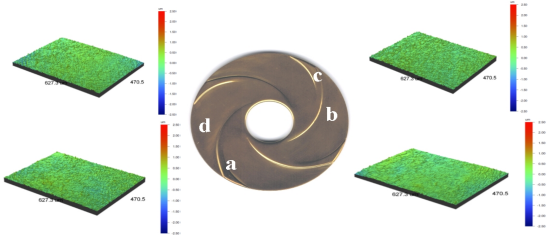

FIG. 3. Surface roughness of TiN layers on the blood pump elements.

The acute thrombogenicity test confirmed blood clotting freedom inside the pump, including impeller (FIG. 4). The micro emboli were observed at surface technological defects.

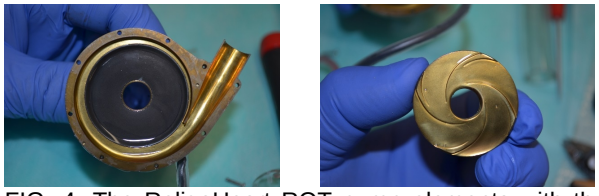

FIG. 4. The ReligaHeart ROT pump elements with the TiN diffusive surface layers after acute thrombogenicity test.

Conclusions

The ReligaHeart ROT prototypes investigations, with the applied TiN+Ti_2N+αTi(N) layers, confirmed the pump and the titanium nitride layers expected surface properties, especially athrombogenic properties in the contact with blood. The further work for human device construction and manufacturing technology development will be carried out.

Acknowledgments

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NOVEL MEASUREMENT METHODS IN THE EVALUATION OF BIOMATERIALS' PROPERTIES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 101]

Introduction

Hernias are serious health problems and if left untreated, can lead to severe complications and even death. One of the most common methods of treatment of hernia is the use of a polymer implant, which allows the "tension free" surgical operation in the course of which the gap is filled with synthetic material (in the form of mesh) instead of manual stitching of tissue's walls. In terms of the application of such implant in the environment, apart from the polymer used and its physical, chemical and mechanical properties, extremely important are structural parameters of the implant. It is expected that the changes in the structural characteristics have impact on a number of product's characteristics, especially surface properties, such as the free surface energy or specific surface area. This in turn influences the final application of the product or material e.g. in catalysts, sorbents, ionic fillers, thermal insulators etc. Unfortunately, harmonized standards of Directive 93/42/EEC [1] do not include this aspect in evaluation and also, at the stage of implants' design standardized methods in this respect are not in use. Mainly biological, chemical and physical properties are included, as the ones having significant impact on the implants' further performance in biological conditions. The ability to assess the basic structural features, such as surface mass, thickness, diameter of the fibres, type of weave and etc. and its correlation to the surface properties like specific surface area or free surface energy are important elements in the design of a medical devices and should be standardized, as the literature confirms the important influence of the structure parameters on the properties of implants. The ability of assessing surface properties will allow predicting the mechanism of action of the implant within the human body. It will also enable to create a more complete picture of the properties of the biomaterial and it will bring also the possibility to predict its mode of action in biological conditions.

Materials and Methods

In the present study an attempt to determine the possibility of using novel measurement techniques for the evaluation of medical implants was made. As a part of examination, three methods of examination were compared - adsorptive porosimetry, inverse gas chromatography and mathematical method of determination of specific surface area. In the frame of the presented work, two kinds of hernia meshes, commonly available on the market, were examined. The monofilament hernia meshes are made of polypropylene and are intended for medical use (implantable materials with over 30 days of use within the body). Basic structural characteristics, such as surface mass were tested in accordance with applicable standards – examination was performed in accordance with PN-EN 29073-1: 199. The surface mass determination was carried out in air-conditioned laboratory, at normal climatic conditions, that is T = 20°C, RH = 65%. Thickness of the knitted fabrics was measured in accordance with PN-EN 5084:1999. The measurements were carried out using the apparatus - Porosimeter ASAP 2020 V3.01 H (adsorptive porosimetry) and Energy Analyzer (SEA) by Surface Measurement Systems (SMS) Instruments (Inverse gas chromatography). Examination of the surface morphology was carried out using high resolution scanning electron microscope NOVA Nanos 230 manufacturing company FEI equipped with an X-ray microanalyzer EDAX Apollo SDD.

Results and Discussion

The obtained results of the specific surface area confirm the hypothesis given at the beginning of work.

TABLE 1. A summary of results obtained by the use mathematical method

	BET Adsorptive porosimetry	BET Inverse gas chromatography	Mathematical method
	m^2/g	m^2/g	m^2/g
Sample I	100,16	17,13	2,114
Sample II	87,60	9,28	1,604

The differences between the results derive from the specificity of the measurement. In the case of analytical methods (adsorptive porosimetry and inverse gas chromatography), the main difference lies in the selected gas for analysis - namely the type and size of the gas molecules used for measurements, which determines the amount of particles deposited on the test surface, which then has an impact on the calculation of specific surface area.

Conclusions

Both of the selected analytical methods are suitable for evaluation not only specific surface area, but also associated characteristics like free surface energy of the sample, acid/base properties, pore size, pore volumes and etc. The third applied method - mathematical one, turned out to be irrelevant to be used in the case of hernia meshes.

Apart from the physical, chemical and mechanical properties of the implant, extremely important are structural and surface parameters. The ability to determine these parameters would allow predicting the mechanism of action of the implant within the human body. Application of these measurement methods in the design of the implant will contribute to shaping the structural properties of implants/medical devices for their final application, as well as shaping their biological characteristics (which are heavily influenced by surface properties). These methods should be incorporated in the design stage of medical devices, as their results are a perfect complement to other methods of evaluation, thereby allowing to a fuller extent determine the expected properties and actions of the implant in a natural environment.

Acknowledgments

The project was finances by the Department of Material and Commodity Sciences and Textile Metrology, Lodz University of Technology, Poland.

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[1] European Council Directive 93/42/EEC of 14 June 1993 concerning medical devices

LITERATURE REVIEW ON ELECTRODEPOSITION AS THE NEW METHOD FOR THE MODIFICATION OF FIBROUS BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 102]

Introduction

The mechanism of electrophoretic deposition comprises deposition of charged particles on the electrode chemicals under the influence of an applied electric field. Theoretical foundations of this process were developed in the nineteenth century, when the Russian scientist Ruess watched the movement of clay particles in the water under the influence of an applied voltage. With the first practical application of this phenomenon we have to deal with in the 40s of the twentieth century (deposition of thorium particles to platinum cathode) and also then firsts patent occurred, while the largest development in this field appeared to be in the 80s and 90s. To date, electrodeposition has found wide use in the preparation of coatings of various types, e.g. strengthening wear resistance, antioxidant, functional coatings for advanced microelectronic devices and solid oxide fuel cells, as well as for the preparation of bioactive coatings and composite materials.

Recently, there is a big increase in interest in the technique especially in industrial applications due to its high versatility (as regards to the choice of base materials and the coating materials), the simplicity of the entire process, low cost of equipment and its maintenance. Among the other advantages we can mention among others short time of deposition, small constraints on the shape of the substrate (carrier to deposit layers) and the fact that, compared to other advanced techniques for deposition, the EPD process can be easily modified for a particular application. For example, deposition can be performed on flat surfaces, cylindrical or any other and this will require only minor changes in the design of electrodes and their positioning [1]. Furthermore, despite the fact that the EPD is a wet process, it ensures control over the thickness and to some extent the morphology of the coating formed by adjusting the deposition time and the applied potential.

The kinetics of electordeposition and characteristics of the produced layers depend on two groups of parameters - associated with the solution for deposition (which consists of the following factors: the particle size and the concentration [2,3], the dielectric constant, [4] conductivity of the solution and the viscosity [5,6], the zeta potential [7-10] and related to the process - physical parameters such as the type of electrodes or the process conditions (e.g. the applied voltage [11,12], the time of deposition [13,14], etc. Unfortunately, the knowledge in shaping the properties of the coatings obtained and the kinetics of the process is not well-structured and there is a lack of systematic scientific description of these issues.

A reasonable is also to conclude that electrodeposition of various types of coatings on metal substrates is relatively well established technique compared to the use EPD for surface modification of fibrous biomaterials, the structure and properties of which (mechanical, thermal, electrical etc.) are significantly different from those mentioned hereinabove. A major limitation in the case of fibrous materials is that most of them have poor conductivity, which often precludes the use of electrophoretic deposition. In contrast, a number of advantages of various types of fibrous structures (e.g. porous structure, flexibility, possibility for obtaining composite structures etc.) caused a significant increase of interest in these carriers and methods of their potential modifications [15].

The work aims at presenting the theoretical review of the current state of the art in the area of the electrodeposition of ultra-thin polymer layers on fibrous structures.

Acknowledgments

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BIOACTIVE SILICA ENRICHED HAp COATING ON TITANIUM WITH NANOPATTERNED TiO_2 INTERMEDIATE LAYER

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[ENGINEERING OF BIOMATERIALS 138 (2016) 103]

Introduction

To enhance the bioactivity and osseointegration of metallic implants, a wide range of technologies has been offered to adjust the surface properties to specific needs. Such treatments include surface anodization [1] and coating with hydroxyapatite (HAp) [2] or enhancement of HAp formation [3]. The nanotubular structure of TiO_2 has been reported to increase significantly the titanium surface area and enhance the formation of HAp and the bond strength between the substrate and the coating [4]. Silica nanoparticles are considered to be an attractive material to improve the biocompatibility and biodegradability of HAp coatings [5]. It was reported that silica nanoparticles have an inhibitory effect on osteoclast differentiation and stimulatory effect on osteoblast differentiation [6].

In the present work, hydrothermal method was used to fabricate highly bioactive HAp coatings enriched with silica nanoparticles on titanium with anodized TiO_2 in the form of nanotubes and u-shapes as an intermediate layer.

Materials and Methods

The TiO_2 nanopatterns were prepared on 14x14 mm^2 titanium plates by standard two electrode anodization at room temperature in potentiostatic mode. The anodizing voltage was kept constant at 50 V during the process. One of the samples from each kind was annealed in oxidizing atmosphere at 600^{o}C for 1 h, whilst the other substrate was left without annealing as a reference sample. In order to produce silica enriched HAp coatings, the hydrothermal process was carried out in an autoclave from stock solution containing: calcium salt hydrate, diammonium phosphate, calcium helating agent and soluble source of silicon.

The morphology and structure of as prepared specimens were characterized using X-ray diffraction, scanning electron microscope, proton induced X-ray emission, infrared and Raman spectroscopy. Changes in surface morphology were examined after 4 days of incubation in simulated body fluid.

Results and Discussion

FIG. 1 shows SEM images of TiO_2 arrays after the hydrothermal process. The research revealed different effect of surface nanopatterning, i.e. nanotubes or u-shapes, on morphology of HAp-SiO_2 coatings. Concerning the ability to induce hydrothermal crystallization of HAp and coating bioactivity, the best results were obtained for annealed nanotubes (B). In the case of unheated substrate with TiO_2 u-shapes, there was no coating formed on its surface after the synthesis (C). Subsequent heating slightly improved the deposition of HAp crystals, although not in order to form homogeneous and continuous coatings (B). The XRD and spectroscopic analysis confirmed the existence of well crystallized HAp and amorphous form of silica nanoparticles in the prepared specimens. The formation of nanosized silica particles within HAp matrix is based on Stober process by hydrolysing monomeric tetraethyl orthosilicate precursors in the presence of ammonia as a catalyst.

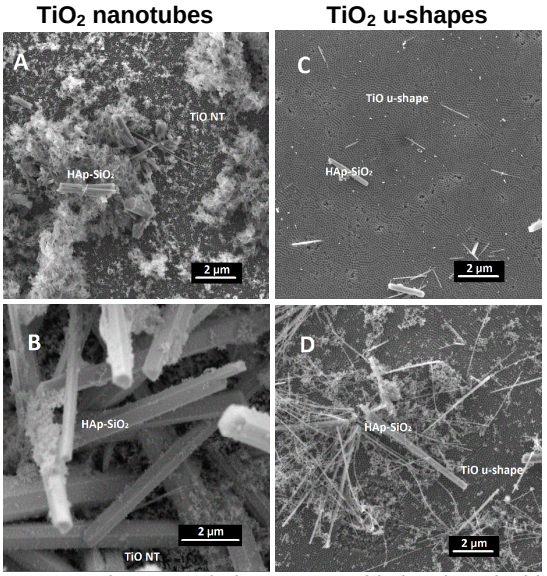

FIG. 1. SEM images of TiO_2 arrays with (B,D) and without thermal treatment at 600^{o}C (A,C) after hydrothermal deposition of HAp coatings enriched with silica nanoparticles.

Conclusions

We demonstrate novel method for synthetizing highly bioactive crystalline HAp coatings enriched with nanoparticles of silica on nanopatterned titanium substrates. We also examined the effect of surface morphology and nature of TiO_2 nanotubes and u-shapes on hydrothermal crystallization of HAp-SiO_2 coatings. The synthesis allows to homogeneously cover the titanium substrate concerning complex geometry, as most of the implants possess. In this study, biodegradable properties of silica nanoparticles (with size ~25 nm) introduced into HAp matrix, can not only enhance nucleation rate of bone-like apatites and stimulate osteoblast differentiation, but also facilitate bone remodeling, being slowly replaced by newly formed tissue. The SBF test revealed high bioactivity of the synthesised coating on annealed TiO_2 nanotubes just after 4 days of immersion.

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EVALUATION OF CORROSION RESISTANCE OF MAGNETRON SPUTTERED DOPED DLC COATINGS WITH USE OF SALT SPRAY TECHNIQUE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 104]

Introduction

Use of the implants, especially metallic ones is connected with several problems such as release of ions or wear debris to the surrounding tissues [1], formation of pathogenic biofilm [2], pure integration with host organism etc. Among the common ways of overcoming those obstacles is the use of various surface finishing methods. Deposition of diamond like carbon (DLC) on biomaterials becomes a promising solution because of the high mechanical properties and biocompatibility of such coatings [3,4]. Further evolution of that approach involves doping of synthetized films in order to tailor its properties in the desired fields. Nevertheless, still one of the key aspects of all the coatings is its uniformity and barrier properties preventing for example corrosion.

The corrosion can lead to the deterioration of structural integrity of the implant and resulting from that reduction of mechanical properties [5], but also invoke dangerous modifications in the body of the patient [6]. Released to the organism corrosion products act both locally, causing severe pathomorphological changes in implant-surrounding tissues, and systemically - affecting organs detoxification.

One of the many approaches enabling to evaluate the corrosion resistance of the materials involves use of salt spray technique. The following study comprised such investigation conducted on novel magnetron sputtered doped DLC coatings deposited on austenitic stainless steel AISI 316 LVM.

Materials and Methods

Deposition of DLC and doped DLC coatings was conducted on cylindrical samples made of AISI 316LVM. Specimens of 16mm in diameter and 6mm of height were mechanically grinded and polished and afterwards ultrasonically cleaned in acetone for 10 minutes.

All the coatings were deposited with use of multi-target DC-RF magnetron sputtering system with two graphite and one target made of pure dopant: Si or Ti (all from Kurt j. Lesker Company). The pure carbon film was deposited at 0.6 Pa pressure in the reaction chamber, 10 sccm of Ar and 200W deposition power (for both graphite targets). The amount of dopant was changed by varying the sputtering RF power on dopant's target.

The corrosion resistance of coated metallic samples and uncoated substrate material was performed in salt spray corrosion S120ip machine (Ascott Analytical Equipment). Before placing the specimens into the salt spray chamber, they were cleaned in deionised water (10 min) and acetone (15 min) with the use of ultrasonic cleaning bath. The corrosive environment was 5% sodium chloride solution sprayed with the flow rate of 20 ml/min. The temperature in the chamber was constant and equal to 37^{o}C. The total time of the corrosion test was 4 weeks. To evaluate the surface properties and possible corrosion symptoms, samples underwent investigation with use of microscopic observation (inverted metallographic microscope Eclipse MA100, Nikon), roughness measurements (contact profilometer, Hommel Tester T1000) and water contact angle measurements (sessile drop technique). Before the corrosion test and in last day of each week of incubation in salt spray chamber.

Results and Discussion

Although the surface appearance greatly changed along with the prolonging exposure to the corrosive environment (dark spots visible on the microscopic images), no signs of the corrosion were observed. The value of Ra parameter for all the samples increased from about 0.1 before contact with salt spray to over 0.4 after 4 weeks of incubation. Nevertheless, there were no pits or hollows visible on the performed roughness profiles. The contact angles for all of the examined cases decreased as the incubation was proceeding. The changes of the surface properties of the examined materials were related to the formation of the salt-based residues and not corrosive processes.

Conclusions

After incubation of samples in 5% salt spray at 37°C for the total period of 4 weeks, no particular signs of corrosion were observed on any type of specimen .

Acknowledgments

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BIOLOGICAL EVALUATION AND SURFACE PROPERTIES OF Ti-DLC COATINGS DEPOSITED BY MAGNETRON SPUTTERING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 105]

Abstract

During the last few decades, a growing demand for medical implants may be observed on the market. This is a consequence of both the increasing number of people suffering from disabilities as well as technological development. As a result of growing number of trauma injuries, orthopaedic and bone implants are one of the branches of the medical device industry showing the fastest growth opportunities. However, the commonly applied metallic implants do not exhibit total chemical stability in human body environment and hence, possess relatively poor surface properties [1]. For that reason, even the corrosion resistant metals may release degradation products and cause adverse biological response[1]. Consequently, surface modifications of metallic implants which enhance the biological response of the human body towards the surface of the implant are recently gaining a lot of interest.

One of the most extensively studied solutions include the application of diamond-like carbon (DLC) coatings which exhibit a combination of highly desirable properties in the context of biomedical applications [2]. Furthermore, the properties of DLC coatings, such as cell behaviour and body reaction towards its surface, may be further improved by doping with various elements [3]. Therefore, the modified DLC coatings are nowadays extensively researched in terms of their possible medical applications.

In the case of orthopaedic implants the enhancement of the osseointegration process is highly desirable in order to assure the proper bone-healing, what according to the literature may be achieved by the addition of titanium (Ti). The incorporation of Ti into the DLC matrix does not only promote the bone marrow cells proliferation, but also simultaneously reduces the activity of the osteoclast-like cells as indicated by Shroeder et al. [4]. Similarly, also Thorwarth et al. demonstrated that carbon coatings containing TiO exhibit promising results concerning the proliferation and differentiation of human osteoblasts [5].

Nevertheless, in spite of the numerous studies considering the biological behaviour of Ti-incorporated DLC coatings, there is lack of conclusive reports considering the biological applications of coatings deposited by a magnetron sputtering technique. Taking this into consideration, a complex biological evaluation of Ti-DLC coatings followed by their surface characteristics was performed, since surface properties have a direct role in different post-implantation reactions including protein adsorption and cell proliferation [1].

The examined coatings were deposited on two commonly applied metallic biomaterials (AISI 316 LVM steel and Ti6Al7Nb alloy) using a magnetron sputtering technique. The surface characteristics of the deposited Ti-DLC coatings included the analysis of surface morphology (SEM), chemical composition and structure (XPS, FTIR) as well as surface wettability and surface free energy (sessile drop technique and Owens-Wendt's model). The biological assessment of the deposited coatings was based on two complementary cell proliferation and viability assays (LIVE/DEAD and XTT test) performed with the use of two different cell lines, i.e. endothelial cells line EA.hy926 and osteoblast-like cells line Saos-2.

The obtained results allowed to check the influence of titanium on the biological response of two different cell lines towards the Ti-DLC coatings deposited using magnetron sputtering method as well as to correlate the obtained results with the surface properties of the investigated coatings.

Acknowledgments

This research has been supported by the National Centre for Research and Development under the grant no. LIDER/040/707/L-4/12/NCBR/2013 entitled „MOBIOMED: Modified BIOmaterials – MEDicine future".

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ALGINATE/CHITOSAN HYBRID MATERIALS LOADED WITH CIPROFLOXACIN

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[ENGINEERING OF BIOMATERIALS 138 (2016) 106]

Introduction

Constantly growing bacteria drug resistance, connected with the possibility of formation by them multicellular community (biofilms), is one of the most serious global public health problems and a huge threat to modern medicine. For the past two decades, biopolymers and among them alginate (AL) and chitosan (CS) were the subject of the intense research related to various medical applications such as materials for regenerative medicine and tissue engineering, nevertheless, as well for inhibition of bacterial biofilm formation [1-3].
Both AL and CS are well-known because of their biocompatibility, biodegradability, non-antigenicity, nontoxicity under the normal physiological conditions, as well as muco- and bioadhesiveness [1]. Thus, they are ideal candidates for preparation of novel biomaterials. Alginate/chitosan formulations (*i.e.* spheres, capsules, core-shell composites, fibers, *etc.*) have been proposed in particular for drug delivery systems. Such biopolymer-based drug carriers offer an alternative approach to effective and controlled delivery of many drugs (*e.g.*, antibiotics), even with improvement of their pharmacological and therapeutic properties [4].

Materials and Methods

Ciprofloxacin-loaded alginate beads (AL_CP) were prepared by adopting the method of Silva *et al.* based on emulsification/internal gelation with some modifications [5]. Freeze-dried alginate cores were coated with chitosan, and subsequently cross-linked with sodium tripolyphosphate (AL_CP_CS).
Alginate-based electrospun fibers loaded with ciprofloxacin hydrochloride were fabricated with application of an Yflow 2.2 D500 electrospinner. After electrospinning fibers were stabilized in ethanol and ionically crosslinked by calcium ions. As well, alginate fibers loaded with CP were covered by chitosan by application of coaxial setup.
The resulting materials were characterized in detail by application of such techniques as: (*i*) size was studied by nanoparticle tracking analysis (NTA), (*ii*) zeta potential was investigated by dynamic light scattering technique (DLS), (*iii*) the presence of chitosan on the alginate core and interactions between a polymer and antibiotic were confirmed by infrared spectroscopy, (*iv*) morphology of obtained materials was analysed by scanning electron microscopy (SEM), (*v*) drug loading efficiency and cumulative drug release profiles were evaluated with UV-Vis spectrophotometry.

Results and Discussion

Herein, we present a comprehensive study focused on optimization of method of preparation of alginate/chitosan hybrid materials – beads and fibers. Beads were constructed of alginate core and chitosan shell. Sodium alginate electrospun nanofibers were prepared by blending alginate with poly(ethylene oxide). Beads and fibers were loaded with ciprofloxacin, a fluoroquinolone antibiotic widely used for wound healing.
Spherical in shape AL_CP and AL_CP_CS beads with a mean diameter *ca.* 160 and 250 nm for AL_CP and AL_CP_CS, respectively and encapsulation efficiency *ca.* 75 % were successfully synthesized. Cumulative drug release studies revealed the extended over time antibiotic release profiles.
AL fibers were electrospun and coated with CS with co-axial technique application. The resulting fibers exhibited a core–sheath structure, revealed by TEM analysis. The average diameter of the AL_CP_CS fibers was less than 200 nm, depending on the composition and electrospinning parameters.

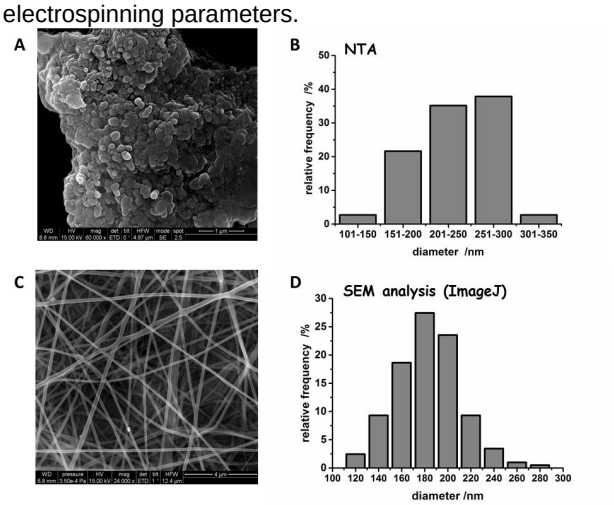

FIG. 1. SEM images and the relative frequency of diameter of obtained materials: alginate loaded with ciprofloxacin beads (A and B) and fibers (C and D), respectively.

Conclusions

In conclusion, four hybrid formulations based on application of biocompatible and biodegradable polymers – alginate and chitosan, both Food and Drug Administration approved biopolymers, were proposed as effective drug delivery systems to fight down multi-drug resistant bacteria. The resulting materials loaded with antibiotic (*i.e.* ciprofloxacin): (*1*) alginate beads, (*2*) alginate beads covered by chitosan, (*3*) alginate fibers and (*4*) alginate fibers covered by chitosan were prepared and characterized.

Acknowledgments

This work was supported by Foundation for Polish Science within POMOST project "*Alginate/chitosan core-shell beads with bioactive functionalities*" (POMOST/2013-7/7).

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PLASMA SURFACE MODIFICATIONS OF POLYMERIC SUBSTRATE FOR APPLICATIONS IN BIOMEDICINE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 107]

Introduction

Polymeric materials have a significant role in medicine, in particular as parts of orthopaedic prosthesis, cardiovascular grafts (*i.e.* artificial heart valves), and artificial organs, *etc.*. Unfortunately, surface properties of these materials such as wear resistance, hardness or wettability do not meet requirements for biomaterials. Therefore, physicochemical properties, as well as biological activity, have to be improved, for instance using thin layer technology [1-3].
Many research groups have obtained coatings on polymeric substrates (*i.e.* polyethylene, polyurethane) using various deposition methods [4-5]. However, in the case of modification of polymeric surfaces for use in implantology, plasma-based techniques are the most suitable ones, especially the chemical vapour deposition (CVD) methods.
In this study, the various types of DLC coatings (as well doped with Si or N atoms) on polyethylene, were deposited under plasma conditions in order to improve mechanical and biological properties of the substrate.

Materials and Methods

Carbon-based coatings were deposited on polyethylene (PE) substrate by RF CVD (*Radio Frequency Chemical Vapour Deposition*) method. Firstly, substrates were functionalization in argon plasma. Then, four experimental series of coatings were obtained: (*i*) single layer: a-C:H or a-CN:H and (*ii*) multil-layer coatings: a-CN:H/a-C:H or a-CN:H/a-SiC:H. All plasma processes were carried out at room temperature and under pressure of gas mixture in reactor chamber below 60 Pa.
Characterization of surfaces were conducted with application of typical engineering methods: (*i*) microstructure and composition of the obtained coatings were determined using scanning electron microscopy (Nova NANO SEM 200, FEI USA) with EDS analyser, (*ii*) topography and atomic structure characterization of the resulting coatings were revealed by AFM and IR-ATR spectroscopic methods, respectively, (*iii*) wettability and surface free energy (SFE) of tested samples were investigated using an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany), (*iv*) hardness and Young modulus of unmodified and modified samples were evaluated by Nanoindenter G200 (Agilent Technologies, MTS Nano Instruments), (*v*) cytotoxicity of the resulting materials was assessed *in vitro* by MTT assay.

Results and Discussion

SEM images confirmed the structure without visible defects and delamination of obtained coatings. In case of sample with a-SiC:H deposition, the EDS analysis revealed the high value of silicon content (*ca.* 27 at. %). This should cause the decrease of internal stresses in the obtained structure and assure low bacterial adhesion.
The most homogenous surfaces were obtained for the deposition of two-layers (a-CN:H/a-SiC:H). The AFM image (FIG. 1B) of two-dimensional surface confirmed the granular and nanometric structure of the deposited coatings.

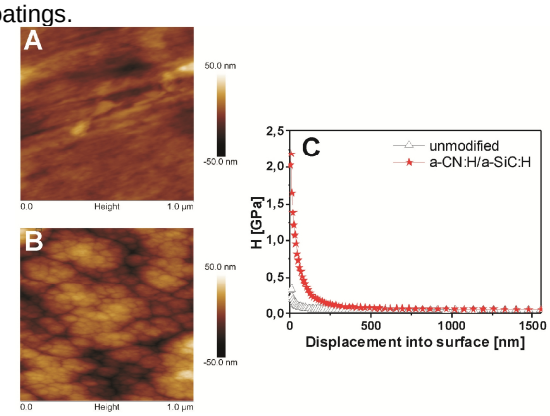

FIG. 1. Surface topography of the PE substrate: A) unmodified surface, B) after a-CN:H and a-SiC:H coatings deposition. C) Hardness profile of the modified PE in comparison with untreated substrate.

All types of plasma modifications significantly improved hardness of the polymeric substrates in comparison with the untreated samples. The highest hardness (*ca.* 2.2 GPa) was observed for the series after the process of modification with two layers deposition (FIG. 1C).
Furthermore, it was demonstrated that for all the tested modified samples with DLC, N-DLC and Si-DLC coatings the characteristic bands' positions in IR spectra were observed at 2750 cm^{-1}÷3050 cm^{-1}, 3300 cm^{-1}÷3400 cm^{-1} and 1250 cm^{-1}, respectively.
For all series of surface modifications after plasma treatment, significant decrease of contact angle was observed, compared to untreated polymeric substrate (up to *ca.* 57^{o}, diiodomethane).
What is noteworthy, for all obtained surface modifications no significant cytotoxicity against osteoblast cell line (MG-63) *in vitro* was observed (less than 20 % reduction of surviving fraction). These preliminary results suggest that the resulting materials may demonstrate a promising biocompatibility.

Conclusions

In conclusion, in this study it was demonstrated that for all plasma-based surface modifications of PE substrate psychochemical properties such as wettability, hardness, and wear resistance were improved. The most significant improvement was observed in case of bilayer deposition of a-CN:H and a-SiC:H coatings. Importantly, all plasma modified samples do not exhibit significant cytotoxicity *in vitro*.

Acknowledgments

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PULSED LASER DEPOSITION OF MAGNESIUM-DOPED CALCIUM PHOSPHATE COATINGS ON POROUS POLYCAPROLACTONE SCAFFOLDS PRODUCED BY RAPID PROTOTYPING

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[ENGINEERING OF BIOMATERIALS 138 (2016) 108]

Introduction

Polycaprolactone (PCL) is a popular polymer in tissue engineering (TE) due to its degradability and low melting point, allowing easy processing into porous 3D structures by methods such as BioPlotting®, a rapid prototyping technique. Thus allows layer-by-layer production of scaffolds with highly defined dimensions and internal architecture (e.g. porosity, pore size). However, poor cell attachment and proliferation on PCL necessitates surface modification. In this study, PCL scaffolds were coated with calcium phosphate (CaP) using pulsed laser deposition (PLD). PLD has been used to coat metallic biomaterials, but PLD coating of polymeric biomaterials remains relatively unexplored. PLD permits doping with elements such as magnesium (Mg) without adversely affecting stability and biocompatibility of CaP coatings. Positive effects of Mg enrichment of inorganic biomaterials *in vitro* have been reported.

Materials and Methods

Porous scaffolds of PCL of dimensions 1 cm x 1 cm, pore side length 0.5 mm were prepared using a Bioscaffolder device (Sys-Eng) as described previously [1] (FIG. 1).

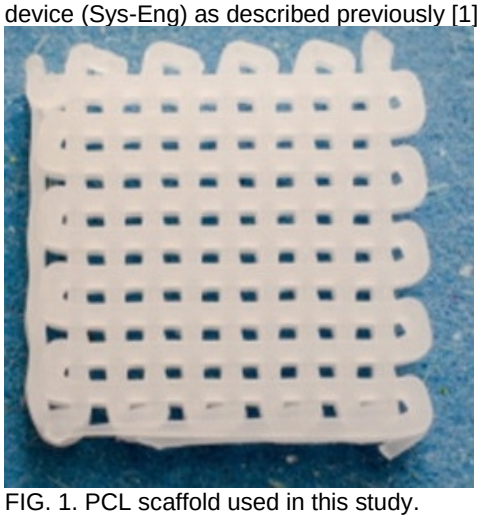

FIG. 1. PCL scaffold used in this study.

Production of hydroxyapatite (HA) and HA+Mg (0.6 w/w %) targets and their deposition by the PLD method was performed as described previously [2]. Three sample groups were prepared: uncoated PCL, PCL coated with CaP and PCL coated with CaP+Mg. Coatings were analysed physicochemically by SEM, EDS and ATR-FTIR and biologically using Saos-2 osteoblast-like cells.

Results and Discussion

SEM images (FIG. 2) showed that coatings were adherent to the substrate. CaP and CaP+Mg coatings were similar, so Mg did not affect coating morphology.

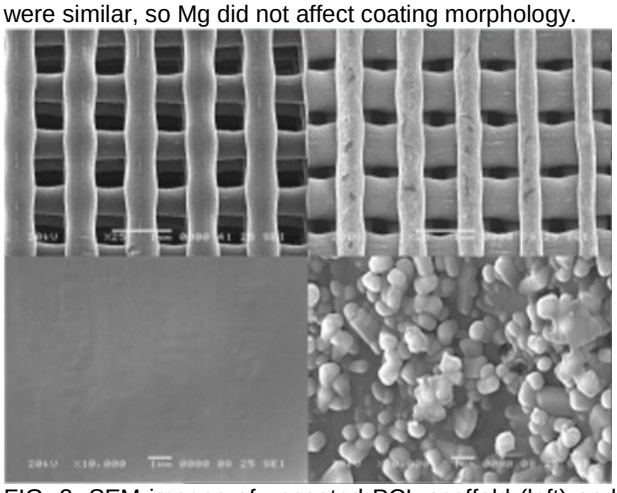

FIG. 2. SEM images of uncoated PCL scaffold (left) and scaffold coated with CaP using PLD (right).

EDS analysis demonstrated the absence of Mg on uncoated scaffolds. CaP+Mg-coated scaffolds contained 0.59±0.13% mass percentage of Mg. ATR-FTIR analysis also proved CaP coating deposition. Spectra of PCL scaffolds coated with CaP and CaP+Mg also showed no obvious differences, demonstrating that magnesium did not significantly influence the type of CaP phase formed.
Confocal microscopy on day 3 after seeding revealed well spread cells homogenously distributed on the sample struts. The overall relative activity of ALP was significantly higher on samples coated with CaP+Mg than on all other samples (FIG. 3).

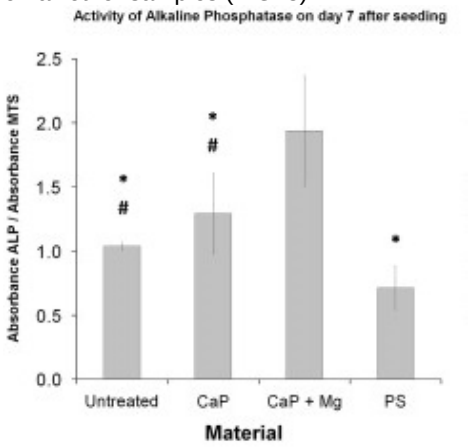

FIG. 3. Activity of alkaline phosphatase (ALP).

Conclusions

CaP and CaP+Mg coatings were successfully deposited by PLD at room temperature on porous PCL scaffolds fabricated by rapid prototyping. All PCL scaffolds supported Saos-2 cell attachment and growth. Activity of the early osteogenic differentiation marker ALP was highest on samples coated with CaP+Mg. The results show that CaP coatings can improve behaviour of bone cells seeded on PCL with a view to bone TE.

Acknowledgments

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BIOINSPIRED, BIOMIMETIC, DOUBLE-ENZYMATIC MINERALIZATION OF HYDROGELS FOR BONE REGENERATION WITH CALCIUM CARBONATE

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[ENGINEERING OF BIOMATERIALS 138 (2016) 109]

Introduction

Hydrogels are popular materials for tissue regeneration due to several advantages, which include the ease of incorporation of biologically active substances such as enzymes. Hydrogel mineralization is desirable for bone regeneration. Mineralization with calcium carbonate ($CaCO_3$) is a promising approach, and has led to superior bone healing *in vivo*. In this study, hydrogels of Gellan Gum (GG), a biocompatible polysaccharide, were mineralized biomimetically using a double enzymatic approach. The enzymes urease and carbonic anhydrase (CA) were incorporated in GG hydrogels. Urease and CA are used by bacteria and marine invertebrates, respectively, to cause mineralization with $CaCO_3$. Hydrogels were then incubated in a mineralization solution containing enzyme substrate (urea) and calcium ions. Urease converts urea to ammonia, which raises pH, and carbon dioxide (CO_2). CA catalyses the reaction of CO_2 with water to form bicarbonate ions, which in turn undergoes deprotonation to form carbonate ions. Subsequently, carbonate ions react with calcium ions to form insoluble $CaCO_3$ inside the hydrogel (FIG. 1).

Urease-catalysed reaction

$$\text{Urea} + 2H_2O \rightarrow 2NH_3 + CO_2$$

Formation of $CaCO_3$ from CO_2 and H_2O

$$CO_2\,(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_{3^-} \leftrightarrow H^+ + CO_3^{2-}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \text{precipitates}$$

FIG.1. Process of enzymatic mineralization by urease.

Materials and Methods

GG hydrogel discs were incubated in 50 mg/ml urease solution containing 0, 0.625, 1.25 or 2.5 mg/ml CA for 1 h to allow the enzyme to diffuse into the hydrogel. Subsequently, discs were immersed in mineralization solution containing 0.27 M $CaCl_2$ and 0.17 M urea as applied by Rauner *et al* [1]. Physicochemical characterization was performed by measurement of dry mass percentage, defined as (Wd / Ww) x 100 where Wd is the weight after drying, Ww is the weight in the wet state before drying, which served as a measure of mineralization. ICP-OES, FTIR and XRD and compressive testing were also performed. MC3T3-E1 osteoblast-like cells were used for biological testing with the AlamarBlue assay and SEM.

Results and Discussion

All hydrogels containing both urease and CA were mineralized more strongly (FIG. 2) and were stiffer than hydrogels which only contained CA. $CaCO_3$ formed was appeared to be predominantly calcite. Autoclaving did not significantly decrease compression strength. Osteoblast-like cell proliferation after 1d, 3d and 8d was not hindered by mineralization with $CaCO_3$. Cell spreading after 8 d was superior on mineralized hydrogels (FIG. 3).

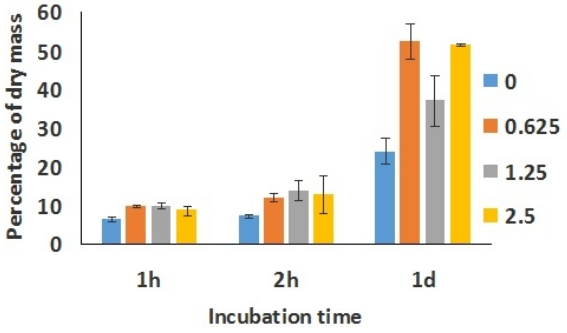

FIG. 2. Dry mass percentage of GG hydrogels preincubated in 50 mg/ml urease with differing carbonic anhydrase concentrations (mg/ml).

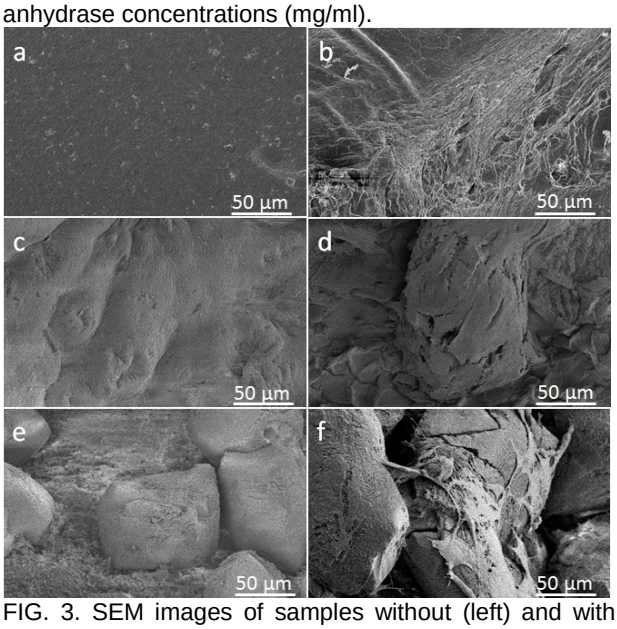

FIG. 3. SEM images of samples without (left) and with (right) MC3T3-E1 osteoblast-like cells 8 d after seeding on unmineralized hydrogels (GG, a&b), hydrogels mineralized for 1 d using 50 mg/ml urease (U, c&d) and using 50 mg/ml urease and 2.5 mg/ml CA (U+CA, e&f).

Conclusions

Double-enzymatic mineralization led to a higher amount of $CaCO_3$ in hydrogels and mineralization did not hinder cell proliferation.

Acknowledgments

FWO and BOF UGent (both Belgium) and the ERA-Net Rus Plus project “Intelbiocomp” are acknowledged.

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ENZYMATIC, UREASE-MEDIATED MINERALIZATION OF GELLAN GUM HYDROGEL WITH CALCIUM CARBONATE, MAGNESIUM-ENRICHED CALCIUM CARBONATE AND MAGNESIUM CARBONATE FOR BONE REGENERATION APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 110]

Introduction

Mineralization of hydrogel biomaterials is considered desirable to improve their suitability as materials for bone regeneration. Calcium carbonate ($CaCO_3$) has been successfully applied as a bone regeneration material, but hydrogel-$CaCO_3$ composites have received less attention. Magnesium (Mg) has been used as a component of calcium phosphate biomaterials to stimulate bone-forming cell adhesion and proliferation and bone regeneration *in vivo*, but its effect as a component of carbonate–based biomaterials remains uninvestigated. In this study, gellan gum (GG) hydrogels were mineralized enzymatically using urease [1] with $CaCO_3$, Mg-enriched $CaCO_3$ and magnesium carbonate to generate composites for bone regeneration.

Materials and Methods

Hydrogels loaded with the enzyme urease (50 mg/ml) were mineralized by incubation in mineralization media denoted as UA, UB, UC, UD and UE (TABLE 1). Mineralized hydrogels were characterized physiochemically by FTIR, XRD, SEM, TGA, ICP-OES and compressive testing, and biologically using MC3T3-E1 osteoblast-like cells.

TABLE 1. Composition of mineralization media UA-UE.

Medium	Concentration		
	$CaCl_2$ (M)	$MgCl_2$ (M)	urea (M)
UA	0.27	0	0.17
UB	0.0675	0.2025	0.17
UC	0.135	0.135	0.17
UD	0.2025	0.0675	0.17
UE	0.025	0.27	0.17

Results and Discussion

Increasing Mg concentration decreased mineral crystallinity. At low Mg concentrations calcite (C) was formed, while at higher concentrations magnesian calcite (MC) was formed (FIG. 1). Hydromagnesite ($Mg_5(CO_3)_4(OH)_2{\cdot}4H_2O$) (HM) formed at high magnesium concentration in the absence of calcium (Ca). Amount of mineral formed and compressive strength decreased with increasing Mg concentration in the mineralization medium. Ca:Mg elemental ratio in the mineral formed was higher than in the respective mineralization media. Mineralization of hydrogels with C or MC promoted cell adhesion and growth, while mineralization with HM led to higher cytotoxicity (FIG. 2).

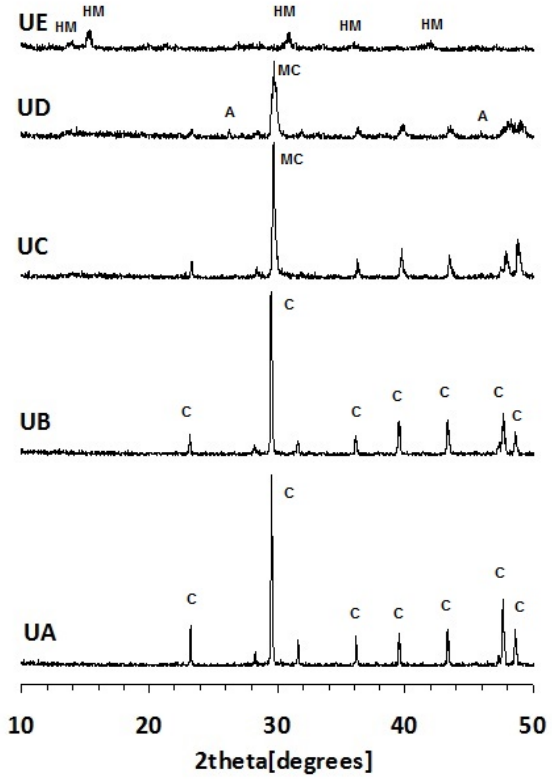

FIG. 1. XRD analysis post-mineralization. A: aragonite; C: calcite; HM: hydromagnesite; MC: magnesian calcite.

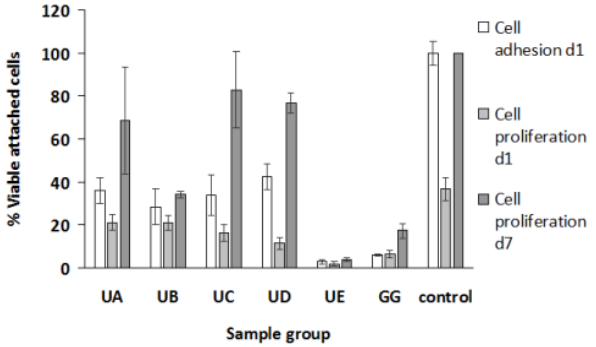

FIG. 2. %viable cells relative to control (tissue culture polystyrene) on day 1 (adhesion) or day 7 (proliferation).

Conclusions

Enzymatic mineralization of GG hydrogels with $CaCO_3$ in the form of calcite successfully reinforced hydrogels and promoted osteoblast-like cell adhesion and growth, but magnesium enrichment had no definite positive effect.

Acknowledgments

T.E.L.D. and A.G.S. thank the Belgian organizations FWO and BOF of Ghent University for support.

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NOVEL INJECTABLE, SELF-GELLING HYDROGEL-MICROPARTICLE COMPOSITES FOR BONE REGENERATION CONSISTING OF GELLAN GUM AND CALCIUM AND MAGNESIUM CARBONATE MICROPARTICLES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 111]

Introduction

The suitability of hydrogel biomaterials for bone regeneration can be improved by incorporation of an inorganic phase in particle form, thus maintaining hydrogel injectability. In this study, carbonate microparticles containing different amounts of calcium (Ca) and magnesium (Mg) were added to solutions of the anionic polysaccharide gellan gum (GG) to crosslink GG by release of Ca^{2+} and Mg^{2+} from microparticles and thereby induce formation of hydrogel-microparticle composites. It was hypothesized that increasing Mg content of microparticles would promote GG hydrogel formation. The effect of Mg incorporation on cytocompatibility and cell growth was also studied.

Materials and Methods

Microparticles were formed by mixing Ca^{2+} and Mg^{2+} and CO_3^{2-} ions (TABLE 1). Microparticle groups A-E were characterized physiochemically by FTIR, XRD, Raman, SEM, TEM, SAED and ICP-OES and subsequently mixed with GG solution to form hydrogel-microparticle composites, which were characterized by µCT, rheometry and biologically using MG63 osteoblast-like cells.

Results and Discussion

The elemental Ca:Mg ratio in the mineral formed was similar to the Ca:Mg ratio of the ions added. In the absence of Mg, vaterite was formed. At low Mg content, calcium magnesium carbonate (CMC) was formed (FIG. 1). Increasing the Mg content further caused formation of amorphous mineral. Microparticles of vaterite and CMC did not induce GG hydrogel formation, but addition of Mg-richer amorphous microparticles induced gelation (FIG. 2) within 20 minutes. Microparticles were dispersed homogeneously (FIG. 3.) Cells were cultured in eluate from composites and on the composites themselves. All composites were cytocompatible. Cell growth was highest on composites containing particle group C.

TABLE 1. Concentrations of solutions used in this study. Equal volumes of Ca^{2+}/Mg^{2+} solution and CO_3^{2-} solution were mixed to form microparticles.

Micro-particle group	*Conc. in Ca^{2+}/Mg^{2+} solution*		*Conc. in CO_3^{2-} solution*
	$CaCl_2$	*$MgCl_2$*	*Na_2CO_3*
	(M)	*(M)*	*(M)*
A	0.333	0	0.333
B	0.250	0.083	0.333
C	0.167	0.167	0.333
D	0.083	0.250	0.333
E	0	0.333	0.333

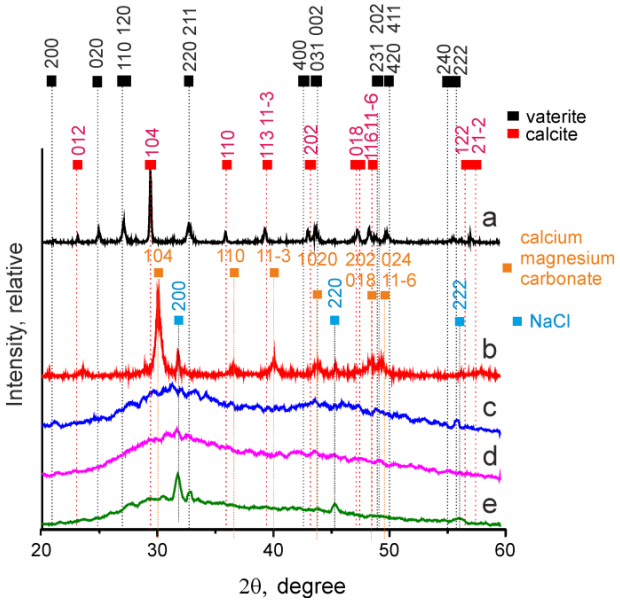

FIG. 1. XRD analysis of microparticles.

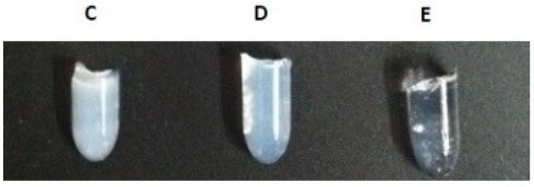

FIG. 2. Images of hydrogel-microparticle composites.

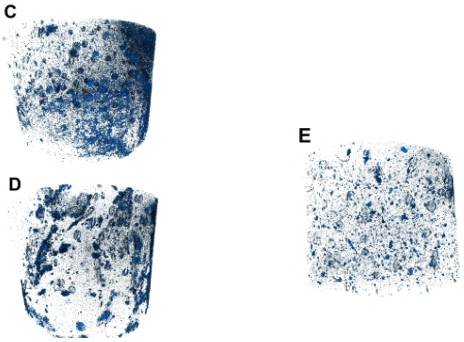

FIG. 3. µCT images of hydrogel-microparticle composites.

Conclusions

Carbonate microparticles containing a sufficient amount of Mg induce GG hydrogel formation, resulting in injectable, cytocompatible composites.

Acknowledgments

FWO, BOF UGent (both Belgium) and the ERA-Net Rus Plus project “Intelbiocomp” are acknowledged.

EFFECTS OF TEMPERATURE ON MECHANICAL BEHAVIOR OF POLY(LACTIC ACID)

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[ENGINEERING OF BIOMATERIALS 138 (2016) 112]

Introduction

PLA is a bioabsorbable, biocompatible, and biodegradable polymer widely used in medical applications [1-5].

The objective of this work is two fold: (i) to present results of experimental investigation of the mechanical behavior of PLA in uniaxial tensile loading–unloading tests, relaxation tests under tension, and relaxation tests under retraction at temperatures in the interval between room temperature and 50ºC.

Materials and Methods

Poly(lactic acid) PLA Polymer 4042D was purchased from Nature Works LLC (Minnetonka, USA). Dumbbell specimens for tensile tests (ASTM standard D-638) were molded by using injection-molding machine.

To evaluate the glass transition temperature Tg and to assess degree of crystallinity K, DSC (differential scanning calorimetry) measurements were performed. The glass transition and melting temperatures of PLA correspond to Tg=63ºC and Tm=155ºC, respectively. Mechanical tests were conducted by means of universal testing machine Instron-5568. The experimental program involved four series of tests at temperatures T=23, 30, 35, 40, 45, and 50ºC.

Results and Discussion

The first series involved loading–unloading tests with cross-head speed 1 mm/min. In each test, a specimen was stretched up to maximum strain ε_{max}=0.015, and unloading down to minimum stress σ_{min}=1 MPa. Observations are reported in FIG. 1. The following conclusions are drawn: (i) the effect of temperature on the mechanical response is relatively weak at T≤35ºC and becomes noticeable at T>35ºC; (ii) in the low-temperature region (T≤35ºC), loading and unloading paths of the stress–strain diagrams are close to each other, and residual strain ε_{min} is small; (iii) in the high-temperature region (T>35ºC), loading and unloading paths differ pronouncedly, and residual strain becomes comparable with ε_{max}; (iv) with an increase in temperature, maximum stress under stretching σ_{max} decreases.

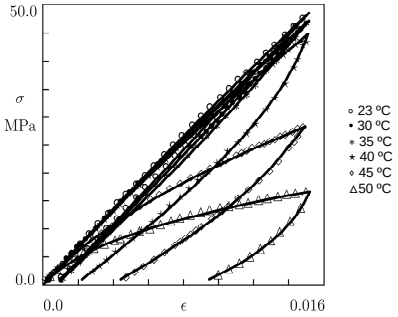

FIG. 1. Stress σ versus strain ε. Experimental data in loading–unloading tests at various temperatures.

The other series involved short-term relaxation tests under tension. In each test, a specimen was stretched with cross-head speed 1 mm/min up to maximum strain ε_{max}=0.015. Afterwards, the strain was fixed, and evolution of stress σ with time t was monitored during t_{rel}=20. Observations are reported in FIG. 2. The following conclusions are drawn: (i) at all temperatures T, stress σ decreases monotonically with τ (simple relaxation); (ii) this decay is rather modest in the low-temperature region T≤35 ºC, and becomes pronounced at T>35ºC; (iii) given a relaxation time τ, an increase in T results in a strong decrease in stress.

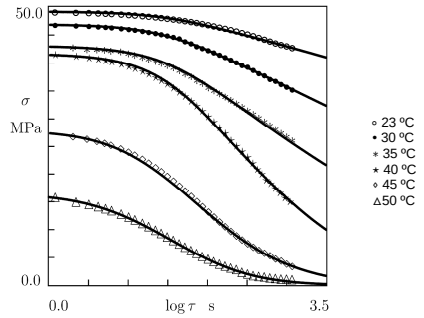

FIG. 2. Stress σ versus relaxation time τ. Symbols: experimental data in relaxation tests under tension at various temperatures TºC (◦ – T=23; • – T=30; ∗ – T=35; ⋆ – T=40; ⋄ – T=45; △ – T=50).

The third series involved short-term relaxation tests on specimens subjected to loading- unloading. In each test, a specimen was stretched with cross-head speed 1 mm/min up to maximum strain ε_{max}=0.015 and retracted with the same cross-head speed down to stress σ_{min}=1 MPa. Experimental data in relaxation tests are presented in FIG. 3 (i) in the low-temperature region (T≤35ºC), stress increases with relaxation time monotonically (inverse relaxation), while (ii) in the high-temperature region (T>35ºC), the dependence σ(τ) becomes non-monotonic (mixed relaxation) with position of the peak decreasing strongly with T.

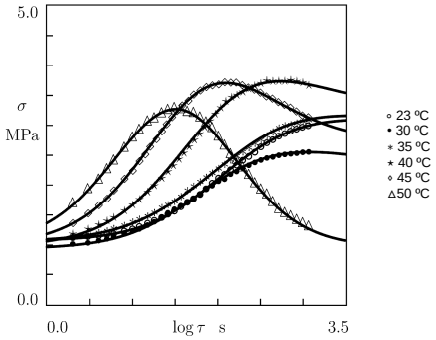

FIG. 3. Stress σ versus relaxation time τ. Symbols: experimental data in relaxation tests under retraction at various temperatures.

Conclusions

From the standpoint of applications, these results indicate that time-dependent properties of PLA are sensitive to temperature, and extrapolation of its mechanical parameters measured at room temperature to the physiological conditions (T=37ºC) should be performed with caution.

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APPLICATION OF ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY IN ANALYSIS OF SiO_2 LAYER USED FOR IMPLANTS IN BONE SURGERY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 113]

Introduction

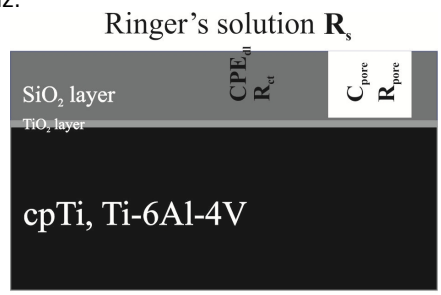
To provide the desired morphological structure and physicochemical properties of the metallic biomaterial manufacturing conditions of the surface layer involving the Si and O_2 were developed [1]. As a substrate material pure titanium cpTi (Grade 4) and Ti6Al7Nb alloy were analyzed. Tested materials met all the recommendations of the normative regarding the chemical composition, structure and mechanical properties (ISO 5832-2, ISO 5832-11), which was verified by preliminary tests [1]. Surface modification of biomaterials was carried out using a low-temperature surface treatment – sol-gel. The application of such surface treatments had no effect on the structure and mechanical properties of the substrate material. In a final step the samples were subjected to steam sterilization.

Materials and Methods

To determine the effect of SiO_2 layer to improve corrosion resistance of cpTi and Ti6Al7Nb impedance test was proposed, which was carried out in Ringer's solution (T = 37 ±1°C, pH = 6,8 ±0,2). The measurements were performed using a potentiostat AutoLab PGSTAT 302N along with a set of electrodes provided with the module FRA2 (Frequency Response Analyser). The measuring system that was used during the study enables research in the frequency range $10^4 \div 10^{-3}$Hz. The voltage amplitude of the sinusoidal excitation signal was 10 mV. In order to assess coating density of the surface layer and also the amount of ions infiltrating from metallic substrate to Ringer solution, metallic ions permeability tests were performed. The amount of ions Ti, Al, Nb and Si that infiltrated to the solution was designated. Each sample was placed for 28 days in 100ml of Ringer solution at the temperature of T = 37±1°C. Metallic ions concentrations were measured with spectrometer JY 2000, by Yobin – Yvon, applying ICP-AES method. The source of induction was plasma torch coupled with generator of frequency 40.68 MHz. When making analytical curve, diluted analytical materials by Merck were applied.

Results and Discussion

The impedance characteristics of the interface: electrode-layer-solution was established through approximation of the experimental EIS data, with the use of a pattern presented in FIG. 1, TABLE 1 and the mathematical model of an equivalent electric circuit. Such model describes drawing of two loops in Nyquist plots. The loop recorded at high frequency, where the diameter of the loop depends on the potential, corresponds to activity of the oxide layer (SiO_2/TiO_2). It is represented by C_{pore} - the capacity of the surface area material having a high degree of surface development and R_{pore} - reflecting the resistance of the electrolyte in the zone of the material. Whereas the low-frequency loop is associated with the oxide layer solution interface, where R_{ct} and CPE_{dl} sub-circuit describes the low-frequency region between 1 and 0,001 Hz.



$$Z_{CPE} = (jwC)^{-a}$$

FIG. 1. Characteristics of the system SiO_2/TiO_2 layer – metallic substrate

TABLE 1. Results of EIS.

Sample	R_s, Ω·cm²	R_{pore}, kΩ·cm²	C_{pore}, µF	R_{ct}, kΩ·cm²	CPE_{dl}	
					Y_0, $\Omega^{-1}cm^{-2}s^{-n}$	n
cpTi	16	9	17	668	0,2587E-4	0,88
cpTi +SiO_2	17	15	29	4485	0,4879E-4	0,96
Ti6Al7Nb	16	7	28	541	0,9029E-5	0,91
Ti6Al7Nb +SiO_2	16	17	33	3369	0,5589E-5	0,92

A mathematical model of the system consisting of Ti alloy- TiO_2/SiO_2 layer-Ringer solution is expressed by the equation (1)

$$Z = R_s + \frac{1}{\frac{1}{R_{pore}} + j\omega c_{pore}} + \frac{1}{\frac{1}{R_{ct}} + Y_0(j\omega)^n} \qquad (1)$$

In turn, the results obtained from the samples bearing a layer of SiO_2 indicate a more compact construction with respect to the passive layers (TiO_2) formed on the titanium substrate. Regardless of the type of substrate, there was no change in the nature of the layer. The resistance of the oxide layer R_{ct} takes large values, reflecting the favorable properties of the samples with a SiO_2 layer. In turn, taking the amount of degradation products infiltrating a Ringer's solution, as an important criterion indicating the level of biocompatibility of a material it should be noted that the SiO_2 layer has the effect of increasing the barrier properties. It has been found that the amount of ions (Ti, Al, Nb) penetrating to a solution of the sample surface covered with a layer of SiO_2 is reduced. This is a positive phenomenon TABLE 2.

TABLE 2. Results of the tests of metallic ion penetration

Sample	Metallic ions infiltration tests, ppm (average value)						
	Ti	SD	Al	SD	Nb	SD	Si
cpTi	0,201	±0,001	-	-	-	-	-
cpTi +SiO_2	0,089	±0,002	-	-	-	-	
Ti6Al7Nb	0,104	±0,001	0,055	±0,001	0,069	±0,002	
Ti6Al7Nb +SiO_2	0,059	±0,001	0,011	±0,002	0,022	±0,001	

Conclusions

Proposing appropriate variants of surface treatment using a sol-gel method has a prospective importance and contributes to the development of technological conditions for precisely formulated parameters of the silica coatings on titanium implants.

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ADHESION OF SiO_2 LAYERS DEPOSITED BY MEANS OF SOL-GEL AND ALD METHODS ON 316LVM STEEL

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[ENGINEERING OF BIOMATERIALS 138 (2016) 114]

Introduction

One of the methods used to improve the hemocompatibility of 316LVM steel is to apply sol-gel and atomic layer deposition (ALD) method to form thin oxide films. Such films have increased hemocompatibility, what greatly reduces the risk of complications related to the intravascular coagulation. One of the most promising layers supposed to get into contact with blood is the oxide SiO_2 characterized by excellent chemical stability and high hemocompatibility [1,2]. The relatively short history of the use of silica layers in biomedical applications transfers into a small amount of literature relating to the physicochemical properties of these layers. The other important issue related to the generation of surface films is providing the proper set of mechanical properties. Therefore, the study involves tests of mechanical properties of SiO_2 films deposited on surfaces of test pieces from 316 LVM steel. In order to assess the mechanical properties of the films generated using sol-gel and ALD method, tests of adhesion of these layers to the metal base were performed. Additionally were carried out of electrochemical tests and surface wettability.

Materials and Methods

The study material was 316 LVM steel 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 electrolytic polishing and chemical passivation and then coating with layer of SiO_2 based on two methods. The SiO_2 layer was applied using the sol-gel and ALD methods. The following process parameters for sol-gel method: v = 3.0 cm/min, T = 430^{o}C, t = 60 min. The silica precursors were: tetraethyl orthosilicate (TEOS), $Si(OC_2H_5)_4$ and tetramethyl orthosilicate (TMOS), $Si(OCH_3)_4$. Other reagents included ethyl alcohol (EtOH) and water. On the other layer studied in this work were grown from Tris(dimethylamino)silan in a low type low-pressure ALD reactor. The deposition process consisted of repeated ALD cycles. The deposition process was carried out at a number of cycles of 2000, at the temperature of 300°C.

One of the methods of verifying the usefulness of this kind of surface modification is testing mechanical properties. A very important aspect concerning the deposition of layers is their adhesion to the metallic substrate, therefore, adhesion of films deposited on 316 LVM steel substrate was tested in this study.

Adhesion was tested using the scratch test, in accordance with the standard. The test was based on generating a scratch using a penetrator – the Rockwell diamond cone – with a gradual increase in the normal force loading the penetrator. To evaluate the critical Lc load, the record of acoustic emission signals changes was used, as well as friction force, friction factor and microscopic observations under an optical microscope, which is an integral part of the platform. Tests were carried out using increasing loading force at the range of 0.03÷25N and the following operation parameters: loading speed 10 N/min, speed of the table displacement 10 mm/min, and length of the scratch ~3 mm. 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 [3]. 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 artificial plasma with the chemical composition recommended by the standard at the temperature T = 37±1°C, and pH = 7.0±0.2.

Results and Discussion

As a result of the conducted researches it was concluded, that the use of the SiO_2 layer has a positive influence on the physio-chemical properties compared to the initial state (without layer) regardless of the application method (sol-gel, ALD). The study of the pitting corrosion resistance revealed, that layers deposited by ALD method were characterised by the highest corrosion potential E_{corr} and polarization resistance R_p. These values amounted to E_{corr} = +15mV and R_p = 1075kΩ•cm^2. A similar trend was observed in the surface wettability tests. The highest value of the contact angle (θ_{av}= 91.4º) was observed for the SiO_2 layer samples deposited by ALD method. In this case, the surface had a hydrophobic character, which is advantageous for implants in contact with blood. On the other hand the adhesion tests conducted for presented application methods, showed that the layer deposited by the ALD method is characterized by the highest value of the critical force. These values amounted respectively to Lc =7.8N for ALD method and Lc = 3.5N for sol-gel method.

Conclusions

In summary, studies have shown that the layer of SiO_2 deposited by ALD method is characterized by more favorable mechanical and chemical properties.

Acknowledgments

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INJECTION MOLDED COMPOSITES WITH IRON OXIDE ADDITION

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[ENGINEERING OF BIOMATERIALS 138 (2016) 115]

Introduction
Magnetite particles (Fe_3O_4) due to their physico-chemical properties currently belong to the most interesting materials considered for applications in medicine and biotechnology. The magnetic particles possess very low toxicity on the living cells [1]. They are used, among others, to enhance contrast in magnetic resonance imaging (MRI) or in bone regeneration [2]. Magnetite can also be used to modify polymer matrix of implantation materials [3]. Implants with reproducible shapes and low cost can be manufactured by injection method. Polymer modifying with magnetite have new properties which can be use in magnetic resonance imaging. Furthermore, the addition of magnetite improves the regeneration of bone tissue. This work demonstrates an assessment of thermal properties and also magnetic resonance imaging was tested.

Materials and Methods
Polymer with magnetite was use for injection. Samples were made in the shape of the tensile flat specimen with different concentrations of magnetite: 0,05wt%, 0,10wt%, 0,25wt% and 0,5wt%. The laboratory injection molding machine V-4-S15N Multiplast was use. Obtained materials were investigated by the MRI technique. The different pulse sequences were tested with the aim to find the optimal sequence for imaging of such materials. Each of the specimens was tested in three mutually perpendicular dimensions. The influence of added magnetite particles on polymer matrix were also studied with differential scanning calorimetry (DSC).

Results and Discussion
MRI visualizations allowed to obtain images of the composite samples modified with magnetite Analysis of the images showed that increasing the concentration of magnetite causes more distorted images. For all samples, the best images were obtained with the spin-echo T_2-weighted sequence. Images of the highest quality were obtained for the materials with the smallest amount of modifier (0.01wt%).
Thermal analysis confirmed that there were not significant differences between properties composites modified with magnetite and pure polymer. We noticed slight decrease glass transition temperature.

Conclusions
The performed analysis have shown that there is a direct connection between the amount of magnetic modifier in the composite and the quality of MRI images. It was stated in this research that the critical amount of the modifier is 0,5wt%. Addition of such quantity of the modifier made the images very distorted and disqualified them from medical diagnosis. This means that, if a good quality of image is needed, the amount of the nanomagnetite particles should be much smaller, the best ~0,05wt%.
The addition of magnetite to the polymer matrix may give new properties. In our study addition of magnetite changed properties of the composite such as, magnetic, or thermal. The performed analysis has shown that there is a direct connection between the amount of magnetic modifier in the composite and the quality of MRI images.
Based on the presented results it can be concluded, that the use of the spin-echo T_2-weighted MRI sequence produces the best images of implants with magnetic modifier and can be used for medical diagnosis, but the investigated implants need to have a proper, much less than 0,5wt%, concentration of the nanomagnetite.

Acknowledgments
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SYNTHESIS OF NANOHYDROXYAPATITE USING MICROWAVE ENERGY

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[ENGINEERING OF BIOMATERIALS 138 (2016) 116]

Introduction

Hydroxyapatite (HAp) is a calcium phosphate compound having a chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It is an inorganic component of hard tissues such as bones and teeth, which is responsible for strength and stiffness. It has been used extensively for biomedical applications, because of osteoconductive property and biocompatibility with human body. Hydroxyapatite is using in regenerative medicine e.x. bone implants for regeneration of bone defects.

Materials and Methods

Nanohydroxyapatite was synthesised by using precipitation method in room temperature and hydrothermal synthesis using microwave reactor MSS2. Thanks to the microwave energy we can easily control the grain size of nanoparticles. Obtained nanoparticles were in the range of 8 - 45 nm grain size. Phase purity was measured using X-ray diffraction. Thanks to scanning electron microscopy (SEM) the morphology of produced nanohydroxyapatite was characterized. The density and specific surface area were determinated using helium pycnomerty and BET method.

Results and Discussion

We obtained 6 types of hydroxyapatite with different crystallinity degree and grain size. Wide variety of GoHAP can be used in many applications (e.x. implants, scaffold layers).

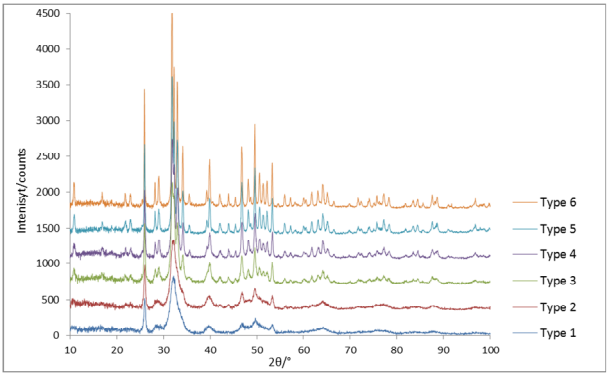

Fig.1. XRD patterns six types of hydroxyapatite.

Conclusions

The Laboratory of Nanostructures is able to synthesize innovative HAp nanoparticles. GoHAP could be a perfect component of the medical implants thanks to good similarity to the natural apatite.

Acknowledgments

The research is realized by the GoIMPLANT: Tough, Strong and Resorbable Orthopaedic Implants (2013-2016) and it founded by M-Era.Net program of The National Centre for Research and Development, co-financed from the European Union, Regional Development Fund.

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CERAMIC-POLYMER COMPOSITES WITH NANO HYDROXYAPATITE FOR ORTHOPEDIC IMPLANTS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 117]

Introduction
Bioactive materials which can support bone ingrowth and osseointegration are common used in orthopedic and dental applications. Bioactive hydroxyapatite can be obtained by the microwave reactor and the high pressure consolidation technology for ceramic materials. The morphology, grain size and specific surface area of the nanopowder can be controlled by the microwave reactor [1].

Materials and Methods
The aim of the GoIMPLANT project was to develop resorbable, tough, strong and biocompatible hybrid composite implants in according to patient's needs. Nano HAP and polymer were connected to solve the HAP brittleness problem. To get better mechanical properties in our laboratory we used combination of GoHAP^{TM} and biocompatible polymers like polylactic acid (PLA) or polycaprolactone (PCL). HAP is one of the inorganic component of hard tissues, which is manufactured in the Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP) and it is called GoHAP^{TM}.

Results and Discussion
Biodegradable composites can be decomposed naturally after a certain period of implantation with their degraded products, which will stay inside the human body. Mechanical and biological performance of composites for implantation depends on the degradation rate. Used degradation medium, the pH and temperature like in human body can determine the degradation test.
Testing the changes of Ca^{2+} concentration of the solution, the conductivity and pH under equilibrium conditions at 37°C PBS was checked once in a week. After that, changes of dimensions and porosity was measured, and shown in SEM microscopy.

Acknowledgments
Research was realized by the "GoIMPLANT" Project and it is founded by M-Era.Net program of The National Centre for Research and Development, co-financed from the European Union, Regional Development Fund.

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EVALUATION OF DIAMOND-LIKE CARBON COATINGS ON PDMS SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 118]

Introduction

In recent years, an increase in the use of carbon coatings in variety of areas including medicine can be observed. Such interest in these coatings is primarily due to their chemical inertness, good corrosion resistance and biocompatibility [1-2]. They are used successfully in the modification of metal and polymer surfaces [3-4]. This work is devoted to research of carbon coatings produced on PDMS polydimethylsiloxane polymer substrates using radio frequency methane plasma (RF PACVD). The investigation was aimed to determine the influence of the thickness of the substrate and RF power on the properties of synthesized coatings.

Materials and Methods

In this work, PDMS from Dow Cornning Inc. company, under the name of Sylgard 184 was used. Before the modification, the substrates were cleaned in ultrasonic cleaner in isopropyl alcohol bath for 10 min and then dried using compressed air, and placed onto the water cooled RF electrode in plasma-chemical reactor. The modifications of PDMS substrates were conducted using radio frequency plasma assisted chemical vapour deposition (RF PACVD) method. The chemical structure was determined using the inVia confocal micro-Raman (Renishaw) and Nicolet IS50 FTIR spectrometers. Dry sliding friction and wear tests were conducted on T-11M tribometer working in ball-on-disc configuration. As the counter sample, commercially available ZrO_2 balls 6.35 mm in diameter were used.

Results and Discussion

Optical microscopy results of PDMS substrates before and after the modification made it possible to determine that the plasma treated PDMS surface is more developed, and takes the form of “wrinkles”. The smallest surface irregularities were observed for carbon coatings synthesized with the RF power of 900 W. In addition, a tendency to increase the surface irregularities with decreasing the PDMS substrate thickness was observed.
The results of Raman spectroscopy primarily were used to determine the chemical structure of carbon coatings in the preliminary investigation. Based on the calculated ID/IG ratios, for each carbon coating, it was determined that it changes depending on the RF power – initially it decreases between 100 and 500 W, and subsequently increases between 500 and 900 W.
The highest concentration of C-C sp^3 hybridized carbon bonds contains the coating synthesized under RF power of 500 W, whereas the lowest was observed for coating synthesized under the RF power of 900 W. Further Raman spectra analysis conducted on samples with different thickness have shown, that in the case of RF power of 300 and 500 W the thickness of the substrate has no significant influence on the shape of registered Raman spectra. The FTIR spectroscopy made it possible to characterize the chemical bonds present on the surface of PDMS substrates and carbon coatings as well. Based on the FTIR spectra in the range between 1500 – 500 cm^{-1} it was determined, that carbon coating manufactured under RF power of 500 W contained higher concentration of Si-O-Si bonds, compared to these synthesized under RF power of 300 and 900 W. Based on the analysis of spectra in the range between 3000 – 2850 cm^{-1} changes in the concentration of C-C sp^3 bonds were determined. Based on the observations of changes of the coefficient of friction in time under load of 1N, it was concluded, that the result of formation of carbon coatings on the PDMS substrate is decrease of this parameter. The value of coefficient of friction for the carbon coatings was between 1 – 1.5, whereas for the unmodified substrate it was 4.2. The lowest value of the coefficient of friction was obtained for RF power of 500 and 900 W.

Conclusions

The results of conducted research proved the influence of the conditions of synthesis of carbon coatings on their surface morphology, chemical structure and tribological properties. It was determined that the highest concentration of C-C sp^3 bonds was characteristic for coatings synthesized under RF power 500 W, and the lowest for RF power of 900 W. Carbon coatings made it possible to decrease four times the value of the coefficient of friction. The presented results prove the possibility of steering the properties of carbon coatings synthesized on PDMS substrates using varying RF power supplied during the modification. The thickness of the modified polymer substrates does not significantly influence the changes in structure and chemical bonds of DLC coatings.

Acknowledgments

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INFLUENCE OF MICROSTRUCTURE AND CHEMISTRY OF CARBON NANOMATERIALS COATINGS ON NERVE CELLS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 119]

Introduction

Carbon nanomaterials including carbon nanotubes (CNT), carbon nanofibers and graphene possess high potential application for nerve tissue regeneration [1]. Carbon nanomaterials will be able, on the one hand, to prevent the activity of astrocytes and, on the other, to stimulate axon growth and the restoration of synaptic connections. These nanomaterials are proposed as promising candidates for nerve stimulation and regeneration especially thanks to their unusual mechanical and electrical properties and morphology similar to some structure of the nerve tissue [1,2].
Carbon nanomaterials like CNT or graphene despite the fact that are composed of the same element differ in terms of microstructure, structure and chemical reactivity. These properties can have influence on cell response *in vitro* and *in vivo* conditions. The aim of these studies was analysis of microstructure, structure and surface chemistry of carbon nanomaterials on nerve cells response *in vitro* condition.

Materials and Methods

Four types of carbon nanomaterials such as carbon nanotubes without functionalization (MWCNT) and two types of functionalized CNT (MWCNT-F and MWCNT-OH) and graphene oxide (GO) were used to creating of pure coatings and hybrid coatings on the metals' surfaces using EPD technique. Optimizing the EPD process aimed to obtain durable and homogeneous coatings.
Many methods such as infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM) and scanning transmission electron microscope (STEM) were used to analysis chemistry, structure and microstructure of the obtained samples. The contact angle of carbon nanomaterial coatings on metal substrates were also measured by the sessile drop method using an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). The surface energy of the carbon coatings were calculated using the Owens-Wendt method. The *in vitro* studies were carried out on the human neuroblastoma cell line SH-SY5Y.

Results and Discussion

Presence of the chemical groups on carbon nanomaterials strongly depends on functionalization method and types of nanomaterials. The highest hydrophilicity of the obtained coatings were observed for carbon nanotubes oxidized in mixture of sulphuric and nitride acids (MWCNT-F) although the highest concentration of oxygen groups were observed for GO. Hybrid coatings containing both carbon nanotubes and GO possess different chemistry and microstructure (FIG. 1) in comparison with pure carbon nanotubes and GO coatings. The predominant effect on microstructure and chemistry of these samples has presence GO inside coating.
The *in vitro* studies show no cytotoxicity effect for analysed coatings and additionally the SH-SY5Y cells viability after 24h, 48h and 72h was different depending on the physicochemical properties of carbon nanomaterials coatings.

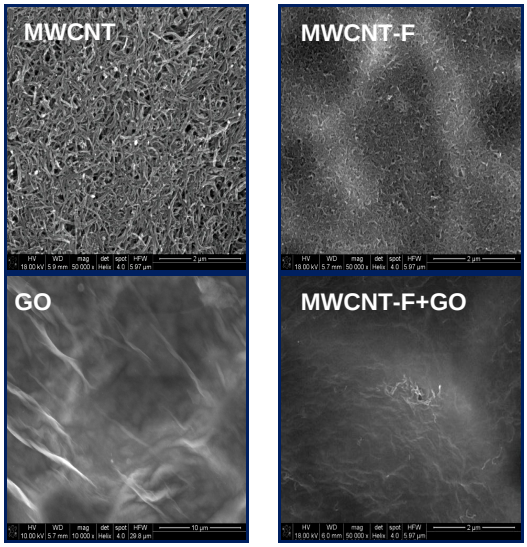

FIG. 1. SEM microstructure of different carbon nanomaterials coatings.

Conclusions

The obtained results indicate that by choosing appropriate type and concentration of the carbon nanoparticles in the coating can be a fairly large extent controlled nerve cell response. To summarize the results obtained are promising and give new insights on issues related to the use of carbon nanomaterials in nerve cells regeneration.

Acknowledgments

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MULTI-PARAMETRIC SPR – *IN VITRO* CHARACTERIZATION METHOD FOR BIOPHARMACEUTICALS AND DRUG DELIVERY SYSTEMS

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[ENGINEERING OF BIOMATERIALS 138 (2016) 120]

Introduction

Surface Plasmon Resonance (SPR) has been used already few decades for label-free characterization of affinity and kinetics of the interaction, like drugs and proteins. New Multi-Parametric Surface Plasmon Resonance (MP-SPR) research instrument extends application range from small molecules also to characterization of biopharmaceuticals and their delivery systems such as real time interactions of proteins, viruses and nanoparticles.

Materials and Methods

Surface sensitive MP-SPR Navi™ (BioNavis, Finland) instrument enables optimization of various analytes (such as nucleotide, peptide, small molecules, antibody, or virus) targeting to different ligands (protein, nucleotide, peptide, receptor, membrane receptor, antibody, or cells). Recently MP-SPR Navi™ was used to characterize virus – peptide interaction for vaccine development [1] as well as to determine adsorption kinetics of gold nanoparticles (50nm) on the self-assembled surface. MP-SPR system allows drug interaction measurements also with biomembranes and uniquely provides conformation information to ensure reliable measurements [2]. Real time drug release kinetics from micrometers thick polymer film for controlled drug release applications was also determined [3].

Results and Discussion

Due to wide angular range measurement MP-SPR enables detection of large shifts in the resonance angle produced by virus or nanoparticle binding and provides precise information for drug delivery studies (FIG. 1). Wide angular range enables also drug release measurements from even micrometres thick polymer films (FIG. 2).

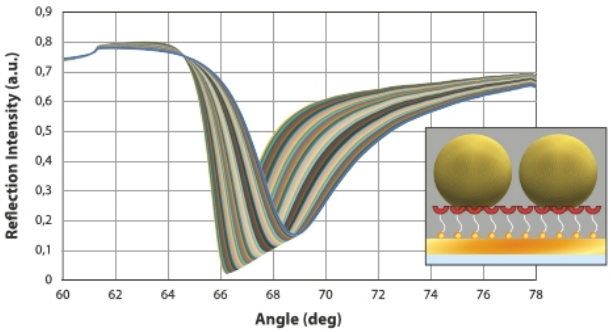

FIG. 1. Gold nanoparticles adsorption kinetic on a self-assembled surface.

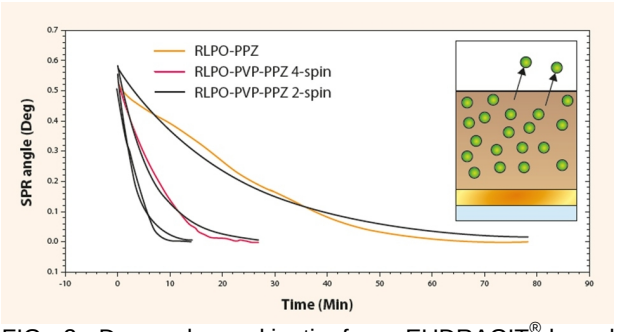

FIG. 2. Drug release kinetic from EUDRAGIT®-based polymer films.

Conclusions

MP-SPR is new *in vitro* characterization tool for biopharmaceuticals enabling development of enhanced targeted drug delivery systems.

Acknowledgments

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DIRECT CRYSTALLIZATION OF SILICATE- PHOSPHATE GLASS FROM $NaMgPO_4$-SiO_2 SYSTEM

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[ENGINEERING OF BIOMATERIALS 138 (2016) 121]

Introduction

In many cases of trauma and injuries to the skeletal system, the particular need for biomaterials with superior properties is required. One of the most attractive materials are bioactive silico-phosphate glasses, which have the ability to form strong chemical bonds with living tissue when they are in contact with biological fluids [1]. Application of glasses as biomaterials is limited mainly due to their very low strength and chemical stability. One of the best ways to improve the mechanical properties of the glasses is to carry out their partial devitrification that allows to obtain glass-crystalline materials [2]. However, this process needs to be highly controlled because the appearance and growth of the crystalline phase may result in loss of bioactivity. In order to fully control the direct crystallization process, it is necessary to know the structure, microstructure and thermal properties of the glassy precursor [3,4]. The most accurate description of microstructure and structure of silico-phosphate glasses provides the opportunity to correctly plan direct crystallization process in order to obtain glass-crystalline materials with premeditated phase composition and crystallite size.

Materials and Methods

The standard sol-gel method was chosen to obtain the materials of the highest possible homogeneity and to reduce the volatility of the individual components. TEOS (SiO_2), $Na_3PO_4 \cdot 12H_2O$ (Na_2O), $Mg(NO_3)_2 \cdot 6H_2O$ (MgO) and H_3PO_4 (P_2O_5) were used to introduce particular oxides. The obtained gels were dried at room temperature (30 days) and then at the temperature of 80°C. To obtain the glassy samples the gels were melted in platinum crucible in the temperature of 1700°C (stabilized for 2 h) and then rapidly cooled. The gradient method was used to perform the direct crystallization process.

The obtained material was examined by thermal methods, X-ray diffractometry, infrared spectroscopy and scanning electron microscopy with the energy-dispersive X-ray spectroscopy compartment. The samples were heated in a gradient furnace for 2 h at the temperature determined on the basis of DSC analysis. In order to identify the type of crystallizing phases the detailed X-ray studies of the materials were carried out.

Results and Discussion

In all studied glasses liquation phenomenon takes place. It means that it was observed glassy spherical droplets (inclusions) in glassy matrix. EDX analysis indicated that chemical composition of inclusions and matrix is different, therefore they must also have different crystallization temperature [5].

The liquation phenomenon seems to be beneficial as it may help to limit the problem associated with the uncontrolled growth of crystalline phases during devitrification. Phase boundary between inclusions and matrix should be a barrier, which can limit growth of crystals. FIG. 1 presents DSC curve of 3Mg glass. The crystallization process runs in several stages. This is evidenced by four separated exothermal peaks, what proves that four different phases crystallize.

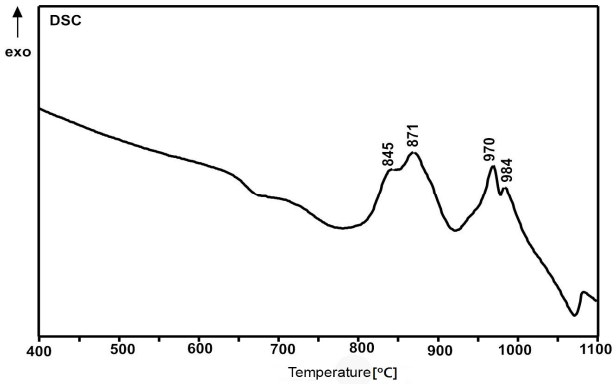

FIG. 1. DSC of the 3Mg glass.

After heating to identify the type of crystallizing phases, obtained materials were analyzed by XRD and MIR methods. Figure 2 presents X-ray pictures of 3Mg glass heated between 800-1000°C. The crystalline phases were α-cristobalite, β-cristobalite, sodium magnesium silicate and sodium magnesium phosphate.

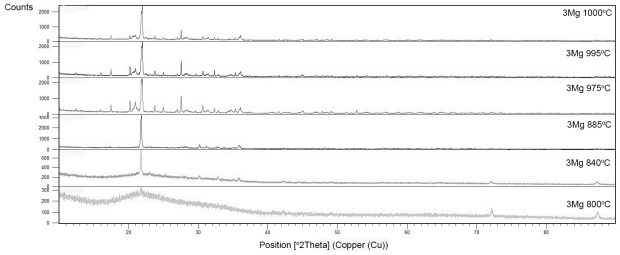

FIG. 2. XRD of the 3Mg glass after annealing at indicated temperatures.

Conclusions

Structural studies of materials obtained after heating in gradient furnace shown that in the first stage cristobalite crystallized and then sodium magnesium silicate and sodium magnesium phosphate. Thus, based on the investigated glasses it is possible to obtain glass–crystalline materials by appropriate selection of crystallization conditions.

Acknowledgments

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BIOACTIVE BLACK GLASSES AND THEIR MODIFICATION WITH CERIUM

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[ENGINEERING OF BIOMATERIALS 138 (2016) 122]

Introduction

Black glasses are interesting novel materials based on amorphous silicon oxycarbide (SiOC). Their structure may be compared to the structure of amorphous silica (v-SiO_2) in which some of the oxygen atoms are replaced by the carbon atoms. Because of the difference in the chemical character between these two elements, two atoms of oxygen are equivalent to one atom of carbon. It is causing the local densification of the structure of the original material and then the improvement of the performances in comparison to the amorphous silica. Stronger network increases the mechanical and chemical resistance, thermal stability and electrical resistivity. In addition, the resistibility to oxidation of the silicon oxycarbide is higher than in the case of silicon carbide. Obtaining of black glasses is challenging due to the crystallization of SiC and controlling of the amount of the free carbon. The most effective way of performing this material is the sol-gel method. It allows the introduction of silicon-carbide bond directly to the precursors by the use of modified alkoxysilanes. The Si-C bond is kept during reactions of hydrolysis and polycondensation and subsequently transferred to the final material. Its presence may be proved after ceramization confirming the efficiency of the proposed method [1]. Properties of black glasses make them perfect materials for protective layers working in hard conditions, for example, in the fuel cells and the catalysis industry and moreover as a biomaterial. The deposition of the SiOC coating may be performed by the dip-coating or EPD methods, which allows the creation of the homogenous and continuous layer on the chosen surface without shape restrictions. The used method of synthesis allows various modifications. One of the most interesting one is the modification with cerium. This rare-earth metal in the form of Ce^{3+} ions may replace Ca^{2+} ions in the hydroxyapatite unit cell. It is due to the similar electronegativity and the ion radius. Moreover, cerium is known as an inhibitor of corrosion and has some antibacterial and fungicidal properties. It is not required that the cerium must be bonded into the glass matrix, it may appear as the nanoparticles of its salts or oxide [2-4].

Materials and Methods

Black glasses were obtained via the sol-gel method. The hydrolysis and polycondensation was performed with the use of methyltriethoxysilane and dimethyldiethoxysilane, which were introduced to the material Si-C band through T and D structural units in the proper ratio. Cerium was introduced to the sol by the addition of cerium nitrate. Obtained sols, with and without Ce, were deposited on the metallic, steel and titanium, with the use of dip-coating method. The chosen speed of the sample movement was established to 20 cm per minute. To obtain the proper thickness of the layer, process was repeated three times. Samples were dried 7 days and then ceramized in 800°C in the protective atmosphere of inert gas – argon. The bioactivity of the material was examined by performing the so-called Kokubo test [5].

The obtained material was examined by thermal methods, such as thermogravimetry and calorimetry, X-ray diffractometry, infrared spectroscopy, Raman spectroscopy and scanning electron microscopy with the energy-dispersive X-ray spectroscopy compartment.

Results and Discussion

The study of the structure proved that the obtained materials were amorphous and contained Si-C bond and may be called black glasses. The addition of the cerium caused the phase separation of the cerium oxide in the form of nanoparticles without changing the matrix material. After performing the Kokubo test, the white residue was discovered. SEM examination showed the spherical forms characteristic for the hydroxyapatite's morphology (FIG. 1). The presence of this calcium phosphate was confirmed with the EDX analysis. FIG. 2 presents SEM microphotograph of the surface of the untreated black glass modified with cerium. It is clearly confirming the similarity between the hydroxyapatite and the cerium oxide nanoparticles with its morphological figures.

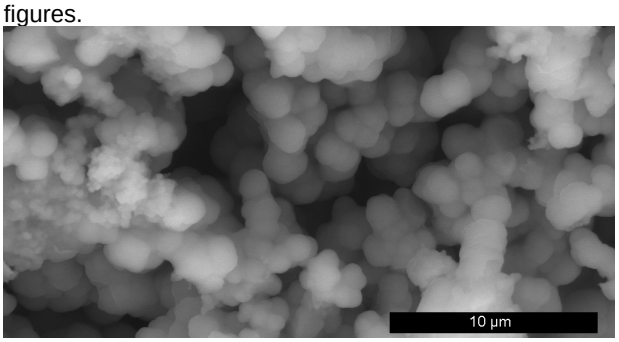

FIG. 1. Hydroxyapatite residue on the surface of the black glass after Kokubo test (SEM microphotograph).

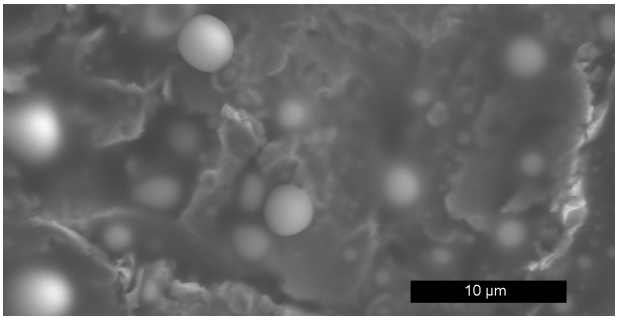

FIG. 2. SEM microphotograph of the surface of the cerium-doped black glass.

Conclusions

The performed experiment and examination proposed promising modification of the known bioactive materials which were black glasses by doping them with cerium. It was done to increase the bioactivity and protective properties and to add new antibacterial and fungicidal characteristics.

Acknowledgments

This work was supported by NCN project "Functional layers of black glasses based on ladder-like silsesquioxanes 2014/15/B/ST8/0282".

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PLASMA MODIFICATION OF NANODIAMOND PARTICLES

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[ENGINEERING OF BIOMATERIALS 138 (2016) 123]

Introduction

This paper concerns the modification of diamond powders (DPP – *diamond powders particles*) in order to achieve the specific physical and chemical properties that would be beneficial for various applications in biomedical engineering. That was the reason why the innovative MW PACVD rotary reactor chamber (MW PACVD – *Microwave Plasma Activated Chemical Vapour Deposition*) was designed and constructed. The material modified in the reactor chamber was tested for potential applications.

Materials and Methods

Currently, there is a great interest in the development of the diamond powder particles (DPP) modification methods, thanks to which they gain new properties [1]. Diamond powders are modified by chemical, mechanical and plasma methods [1,2]. The MW PACVD is one of the methods used for the modification of the DPP. The technology of modifying the DPP by the MW PACVD method, by the use of the rotary reactor chamber, may be much more advantageous in comparison to commonly used methods that use the static reactors. It allows to carry out the modification process in a continuous and cyclic way (through the repeated rotation of the reactor chamber) at the level not reachable for the classical methods. At the same time, it will lower the costs, increase the efficiency and allow for the control of the level of DPP modification.

This paper concerns the basic research that were carried out in the field of the material engineering, more specifically in the technologies of plasma modifications of diamond powders. During the implementation was carried a careful analysis of the physical and chemical processes that occur during the process of DPP modification (first of all, the influence of the rotation of the reactor chamber on the level of the DPP modification). The research carried out are mainly design realizations and experimental studies. These are the original research works conducted in the field of obtaining and modification of the DPP, which make an invaluable contribution to the development of this discipline and, in particular, to the development of plasma methods.

Results and Discussion

The initial concept involved the use of the vacuum chamber (FIG. 1.) equipped with a quartz window (1), the inlet of the reactive gases (6), and the vacuum pump connector. The chamber was supposed to be water cooled and was to be equipped with the system of elements allowing the free rotation of the inner drum (3) e.g. with the use of the stepper motor (7). The basis of the construction would be the typical ECR chamber (electro cyclone), due to that in the construction were predicted such elements as: electromagnets (2) – introducing the excited plasma creative gases (plasma) in the spiral movement directed towards the narrowing of the chamber and the nozzle of reactive gases (6). The quartz window was to be equipped in the main axis of rotation. During the rotation of the drum, the material subjected to the modification is to be located, repeatedly and cyclically, in the area of microwave plasma. The chamber, in contrast to the presented drawing, will have the horizontal orientation.

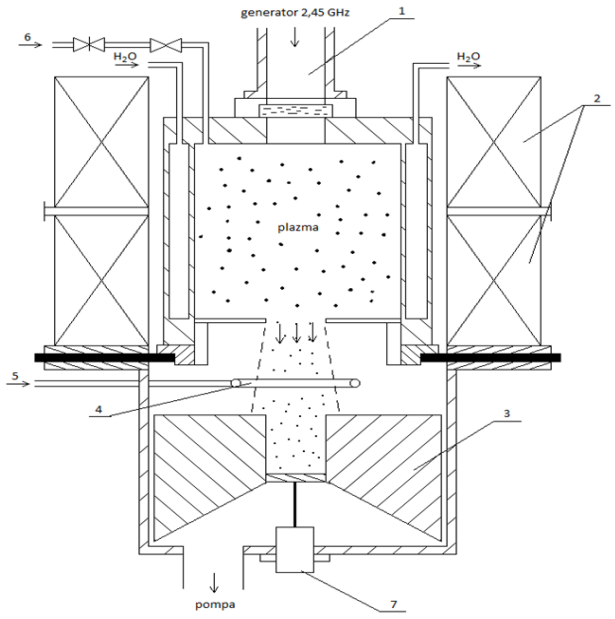

FIG. 1. The concept of MW reactor chamber.

Biological test was carried out by in vivo method on cells of the human blond haemoglobin for all three types of nanodiamond. The images of the optical microscope indicate the following:

- The result of contact of human haemoglobin with not modified nanodiamond powder, in ten minutes is the occurrence of the schistocytosis (decomposition) of erythrocytes.
- The result of contact of human haemoglobin with modified nanodiamond powder, in ten minutes' time no changes are observed.

The analysis of diamond nanopowders using Raman spectroscopy show unnoticeable differences within the analyzed spectrum. It signifies that despite the modification by the MW PACVD + R method, the structure of nanodiamond was fully preserved (no increase in the content of σsp^2 diamond phase providing for overlapping potential partial graphitization of the substrate).

The analysis of diamond nanopowders using FTIR method show small differences in the peaks located on the wavenumber 1419 cm^{-1}, 1636 cm^{-1} and 3424 cm^{-1}, which cause spectacular differences in the biological properties of the modified diamond nanopowders.

Despite the unnoticeable differences in the results of the Raman and small differences characterizing diamond nanopowders by FTIR method, in the biological tests carried out by in vivo method on the human haemoglobin, the results are evident and clearly indicate high biocompatibility of the modified carbon material.

Conclusions

The new knowledge, about the impact of the innovative design of the MW PACVD reactor chamber on the plasma processes occurring in its inside, was acquired during the subsequent experiments with its use.

Acknowledgments

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