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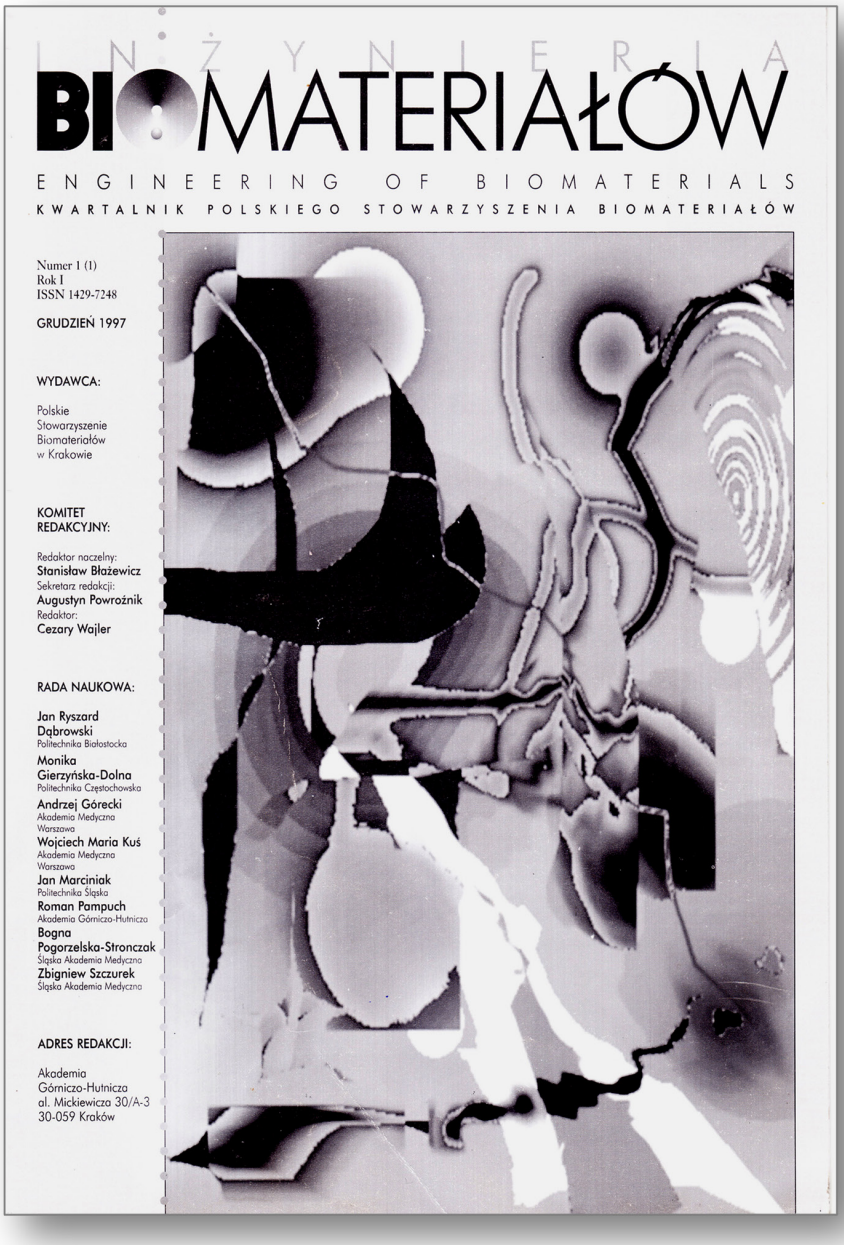

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HEART INJURY AND REPAIR: HOPES AND HYPES OF CELL THERAPIES AND BIOMATERIALS

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During myocardial infarction about a billion of cardiomyocytes (CMs) is lost and the human heart is healed by fibrosis, leading often to heart failure being recently a world-wide epidemics. The lack of regenerative capacity of adult human heart is caused by the property of CMs, which are terminally differentiated cells. However, it has been demonstrated that some CMs can undergo divisions which in adult human heart is estimated to reach 1% of CMs per year. It is considered that this feature is a remnant of effective proliferation of fish and amphibians CMs or the CMs of new-born mammals, which like mice can efficiently heal the heart within first week of life. Accordingly, it is considered that this repairing capacity, lost with adulthood, can be exploited for the purpose of regenerative medicine.

Uncritical belief in the (omni)potency of stem cells (SCs) (or in fact any cell named as "stem" cells), combined with the lack of understanding of the SCs features resulted initially in application of bone marrow-derived cells for treatment of myocardial infarction and then developing heart failure [1]. The results of these first studies, although very much advertised and acclaimed, have been, however, refuted and it has been demonstrated that bone marrow-cells do not have the capacity to differentiate to CMs. However, this did not stop the clinical trials which have been initiated without confirmation of the pre-clinical experiments. This resulted in numerous applications of BM-derived autologous or allogeneic cells which are injected into the failing heart [1,2]. However, accumulating evidence clearly demonstrate that the effectiveness of such a treatment is transient and limited, if any [1]. The refuted claims of differentiation capacity have been then replaced by the paracrine hypothesis, suggesting that the injected cells release the substances which stimulate endogenous heart repair mechanisms. Meanwhile, the persistence of the injected cells in the heart is very limited what questions also the humoral effect.

Nevertheless, these doubtful approaches do not exclude the possibility (although maybe very limited) for stimulating the regeneration of damaged heart. It can be considered that some molecules, active in the early periods of heart development, when human CMs are still capable of division, can be used for stimulation of the proliferation of heart CMs after myocardial infarction. To this end the efficient overexpression of such molecules can activate CMs divisions. Moreover, the pluripotent stem cells, able to differentiate to CMs, are tested for application in heart regeneration. Embryonic SCs (ESC) or induced pluripotent SCs (iPSCs) can now be easily differentiated not only to CMs but also endothelial cells, raising the possibility of effective stimulation of heart regeneration [3-5]. However, achieving real clinical effectiveness will require overcoming the problems of electrical incompatibility of beating CMs and the heart, what creates the danger of arrhythmia after the cells' injection into the organs. This can be potentially solved by delivery of not differentiated CMS, but their precursors, isolated during ESCs or iPSCs differentiation to CMs. However, even if such progenitors or CMs will be generated in the sufficient numbers, they will still face the rapid elimination from the heart after the injection. To this end different scaffolds are being tested, opening the possibility for various biomaterials to be used both for immobilization of the injected cells in the heart and improving their regeneration potentials.

The promise for the future of the regenerative medicine in heart diseases is possible. However, one has to balance the expectations and the possibilities, not offering the hype which are not linked to scientific rationale and experimental evidence.

Acknowledgments

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References

[1] Dulak J, et al,. Adult stem cells: hopes and hypes of regenerative medicine. Acta Biochim Polon, 2015, 62(3):329-37.

[2] Wojakowski W. et al. Effects of intra- coronary delivery of allogenic bone marrow-derived stem cells expressing heme oxygenase-1 on the myocardial reperfusion injury. Thrombosis and Haemostasis, 2012;108:464-75.

[3] Kachamakova-Trojanowska N et al, The real face of endothelial progenitor cells – circulating angiogenic cells as endothelial prognostic marker? Pharmacol Rep., 2015;67(4):793-802.

[4] Kachamakova-Trojanowska N et al. Generation of functional endothelial cells with progenitor-like features from murine induced pluripotent stem cells. Vascul Pharmacol. 2016;86:94-108.

[5] Stepniewski J, et al. Induced pluripotent stem cells as a model for diabetes investigations. Sci Rep. 2015 Feb 26;5:8597. doi: 10.1038/srep08597.

CO-POLYMERIC BIOMATERIALS FOR BONE TISSUE ENGINEERING

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[Engineering of Biomaterials 143 (2017) 6]

Introduction
Biodegradable biomaterial scaffolds are useful tools to conduct tissue development. At the same time biomaterials have an impact on the host immune response. The induced immune response is essential since it can facilitate the healing process. It is therefore important to predict and promote the proper immune response after implantation [1]. The aim of the present study is to synthesize and characterize chitosan-*grafted*-poly(ε-caprolactone) copolymers (CS-*g*-PCL) with PCL contents of 20 wt% and 50 wt% and evaluate (i) their immunomodulatory potential by analyzing the differentiation of primary bone marrow derived macrophages (BMDMs) cultured on copolymeric films, (ii) the osteogenic differentiation potential of pre-osteoblastic cells on copolymeric films, and (iii) the angiogenic potential of human umbilical vein endothelial cells cultured on copolymeric materials.

Materials and Methods
Copolymeric material specimens were synthesized and characterized by scanning electron microscopy (SEM), NMR, FTIR [2]. Cell culture experiments were performed with primary bone marrow derived macrophages (BMDMs), pre-osteoblastic cells MC3T3-E1, and human umbilical vein endothelial cells (HUVECs). Cell viability and proliferation was quantified by means of the PrestoBlue assay. Cell morphology on the copolymers was visualized by SEM and fluorescence confocal microscopy. The osteogenic response was evaluated in vitro by measurement of the alkaline phosphatase activity, collagen production in the ECM, calcium biomineralization by alizarin red staining, and osteogenic gene expression by PCR [3]. For the assessment of the in vitro angiogenic response we quantified the production of Platelet Derived Growth Factor (PDGF BB), a characteristic marker of endothelial "tip cell", which leads the angiogenic sprouting, as well as the expression of angiogenesis-related genes DLL4, VEGFR2, ANGPT2, SPROUTY2, PDGFBB an MMP2 by means of semi-quantitative PCR.

Results and Discussion
We have successfully synthesized novel CS-*g*-PCL copolymers and prepared thin films on glass substrates. *In vitro* experiments of BMDM onto CS-*g*-PCL films have shown a strong cell attachment and good cell proliferation after 7 days in cell culture. Our data from the cytokines secretion detection by ELISA show that the CS-*g*-PCL copolymer significantly decreases the secretion of the inducible levels of pro-inflammatory cytokines IL-12/23 by 31±6%, and thus possesses anti-inflammatory ability. Moreover, this anti-inflammatory action is correlated with the increased chitosan content of the copolymer. In addition, the CS-g-PCL copolymer significantly enhances the production of arginase 1 (Arg1), the hallmark of M2 polarized macrophages, as shown by semiquantitative RT-PCR analysis.

Conclusions
We demonstrate an enhanced osteogenic response of pre-osteoblastic cells on CS-*g*-PCL copolymers and a pronounced angiogenic differentiation potential of human umbilical vein endothelial cells, supporting their potential use as scaffolding materials in vascularized bone tissue engineering.

Acknowledgments
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References
[1] L. Papadimitriou, M. Kaliva *et al*., ACS Biomaterials Science & Engineering (2016) DOI: 10.1021/ acsbiomaterials.6b00440
[2] M. Chatzinikolaidou, M. Kaliva, *et al*., Current Pharmaceutical Design, 20(12) (2014) 2030-2039
[3] K. Terzaki, E. Kalloudi *et al.,* Biofabrication, 5(4) (2013) 045002, doi: 10.1088/1758-5082/5/4/045002

RADIATION FORMATION OF HYDROGEL BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 7]

Abstract

One of indispensable requirements related to biomaterials is their sterility. Ionizing radiation seems to be a very convenient and useful tool to achieve that, especially in relation to polymeric components of medical devices and their packaging. However, in parallel to destruction of living microorganisms radiation treatment causes changes in physicochemical properties of irradiated materials. Both electron beam and gamma radiation used for sterilization may initiate all types of fundamental polymer reactions in biomaterials, i.e. polymerization, degradation, crosslinking, grafting, oxidation, gases formation, etc. The presence of water in irradiated systems enhances the observed changes by indirect effect of radicals formed in water at high yield. Some of such changes are undesirable from the point of view of properties of biomaterials and have to be suppressed, e.g. by introducing into composition of materials selected additives, as it is done for polypropylene syringes or UHMW polyethylene applied for total joint arthroplasty.

On the other hand, such "additional" reactions during terminal sterilization of biomaterials can be utilized for achieve their final, desired properties.

In the last 30 years, our research group has created a number of radiation technologies of polymeric biomaterials. Some of them have been commercialized and are used, modified and developed by other laboratories and companies all over the world, some of them still await industrial investments. The comprehensive review of such biomaterials will be presented including hydrogels dressings, hydrogel systems for induction of childbirth, hydrogel-based dietary product, hydrogel-based hybrid artificial organs, hydrogel implants for intervertebral discs, hydrogel dosimeter for radiotherapy, hydro-nanogels, degradable and/or non-degradable scaffolds for regeneration of peripheral nerve and formation of animal neural tissue in 3D, thermoresponsive surfaces for cultivation of skin cells as well as the method for preservation of biological activity of peptides undergoing radiation sterilization in aqueous solution.

The developed technologies as well as the area of their applications have formed a new direction of research – radiation engineering of polymeric biomaterials – see Report for the IAEA [1].

Conclusions

The above mentioned biomaterials and new methods have been achieved using a typical radiation chemistry methodology and experimental techniques, e.g. sol-gel analysis, molecular weight measurements, pulse radiolysis with spectrophotometric and light scattering detection methods (LSI) as well as specific tests recommended for biomaterials by ISO 10993.

Human-friendly hydrogel systems, due to the rising trend to prolong life span and to improve the results of medical care, seem to be one of the most expected and required products. The unique advantages of radiation technology can be successfully utilized for the preparation of new commercial products, with designed functions that satisfy expectations of patients and physicians. Implants, wound dressings, drug delivery systems, artificial organs, and bioengineering generally are the domains in which radiation formed polymer materials begin to play an increasingly significant role. Despite a great number of investigations on radiation processes which allow for clarification of some mechanisms of reactions and elaboration of some general rules governing those phenomena, there are still some doubts and needs of further studies, both fundamental and applied. Despite many patents devoted to radiation bioengineering there are continuing needs for new products and more sophisticated biomaterials. The use of ionizing radiation in the production of human-friendly products seems to be the most promising way to broaden the range of commercial applications of radiation technology.

Acknowledgments

The author thanks all co-workers and former PhD students who have made a significant contribution to the presented technologies, especially to Prof. P. Ulanski, the first of them and the current head of our Research Team.

References

[1] http://mitr.p.lodz.pl/biomat/old_site/overview.html

ALTERNATIVE BIOCERAMICS FOR REPAIRING JAW BONE DEFECTS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 8]

Historical evolution of bioceramics

Bioceramics have been used as materials of choice for repairing bone defects of the jaws since 60s. The main representative at that era was alumina (Al_2O_3). Next years different types of carbons there also were used for the same reason. In the middle of 80s zirconia (ZrO_2) became a very popular bioceramic in orthopaedics and maxillofacial surgery. Calcium phosphates in general and especially hydroxyapatite played a determent role as jaw bone defect repairing materials, mostly because the negative immune response of human body. Calcium sulphate and trioxide aggregates are nowadays also available for bone defect repairing materials. Recent decades the meanings of biomimetics, nanotechnology and functionalization led research to new paths for the development of combined bioceramics able to mimic the bone microstructure, to present the benefits of the nanoproducts and to be very irritating leading human osteoblasts to produce narrative healthy bone around them. Depending of the purpose they were intended to use, they present proportional properties as they are the mechanical properties, the porosity, the bioactivity and the absorbance. For extended areas of bone loss bioceramics with high mechanical strength, and high percentage of porosity with high porous size distribution were preferable, while in the case of small bone loss more bioactive with balanced absorbance bioceramics were preferable.

Experimental work

A brief report of five experimental studies are going to be presented, the first of which has to do with the fabrication of 3D porous scaffolds with complex geometries using a hydroxy-apatite/chitosan composite. In this work the efficiency of nanohydroxyapatite (nHA/CS) vs. hydroxyl-apatite (HA/CS) was tested (FIG. 1). The second deals with the in vitro evaluation of bioinspired, chitosan based 3D hybrid nanohydroxyapatite scaffolds where a physical proteinic cross-linker extracted from plant gardenia was used. The third presents the structural and mechanical characterization of biphasic α-tricalcium phosphate-nano-hydroxyapatite bone cements, the forth deals with the fabrication of biocements and implants by combination of nanostructured geopolymers and calcium phosphate and the fifth studies the development of 3D scaffolds using a combination of nanohydroxyapatite-carbon nanotubes-biopolymers for promoting bone regeneration. The procedures followed for the fabrication of every combined nanoceramics were different using specific laboratory techniques. From the above-mentioned experimental products, SEM images were received and EDS analysis was performed. Mechanical properties, μCT analysis for porosity 3D profilometry, cell cultures and experiments in animals were also conducted (FIG. 2).

Results and Discussion

Many authors [1-3] have proposed a numerous of techniques and materials combinations for the production of new bioceramics for repairing bone loss in the individual maxillofacial field producing HA nanocrystals and scaffold microstructure quite similar to those of the natural bone.

Experiments revealed a range of porous size distribution starting from microporosity till high porous sizes with satisfactory interconnectivity. New factors in the synthesis of the combined bioceramics can play a decisive role in their biological performance, as for example are genipine as natural cross-linker, the presence of amino acid L-arginine and geopolymers, which assist to viable proliferation of osteoblasts in the produced scaffolds and give to them better mechanical properties.

Experiments with cell cultures and implantation of new bioceramics in animals verified in the most of the cases their beneficial interaction with osteoblasts.

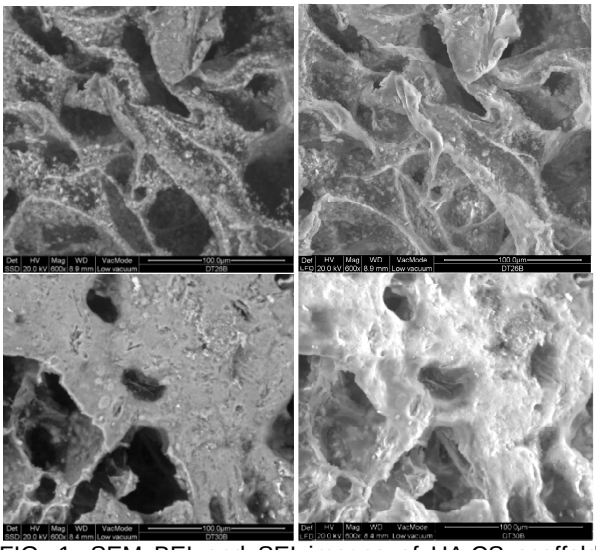

FIG. 1. SEM BEI and SEI images of HA:CS scaffolds (upper row) and nHA:CS scaffolds (lower row).

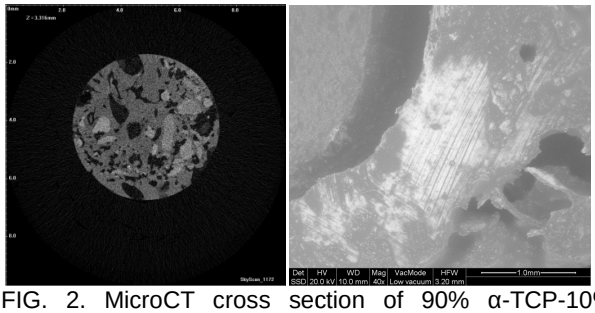

FIG. 2. MicroCT cross section of 90% α-TCP-10% geopolymer, implanted in New Zealand femur and the relevant histological picture.

Conclusions

The conclusions derived of the above-mentioned experiments are that new combinations of bioceramics produced using nanotechnology can improve efficiently the replacement of lost bone with the precondition that the correct fabrication properties and the right surgical procedures will implemented.

References

[1] Zhang, J.; Liu, W.; Schnitzler, V.; Tancet, F.; Bouler, J-M. Calcium phosphate cements for bone substitution: Chemistry, handling and mechanical properties. Acta Biomaterialia 2014, 10(2), 1035-1049.

[2] Thamaraiselvi, T.V.; Rajeswari, S.; Biological Evaluation of Bioceramic Materials- A Review. Trends Biomater. Artif. Organs 2004, 16(1), 9-17.

[3] Daculsi, G.; Laboux, O.; Malard, O.; Weiss, P. Current state of the art of biphasic calcium phosphate bioceramics. J. Mater. Sci. Mater. Med. 2003, 14(3), 195-200.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE SURFACE FREE ENERGY OF VILLACRYL SV4 MATERIAL

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[ENGINEERING OF BIOMATERIALS 143 (2017) 9]

Introduction

The varying environmental conditions of oral cavity may influence on surface properties of dental prosthesis. The high value of surface free energy of dental materials may cause unsuitable processes of bacterial plaque adhesion. An analysis of surface free energy and levels of wettability, cohesive energy, parameter of surface solubility become key aspects in testing of biomaterials [1,3,4].

Materials and Methods

The object of the study was dental acryl Villacryl SV4. The dimensions of samples were: 10.55 (±0.83) x30.10 (±1,10) [mm] and thick 4.75 (±0.21) [mm], all samples were made according to producer instruction. The changes of surface free energy (γ_S - SFE), its components (dispersive - γ_S^d, polar - γ_S^p) parameter of solubility (δ) and cohesive energy (e_c) by using analytical Owens-Wendt (OW) model were estimated. Two measurements liquids: (W) distilled water (Poch S.A.) and (D) diiodomethane (Merck sp.zo.o.) were used. The volume of liquid drop was 0.5 [µl], each test was repeated ten times in room temperature 22 ±1°C. The contact angle values were measured with the use of sessile drop method by the See System computer-based instrument produced with Advex Instruments. The samples were storage in different environments and thus divided into groups (G): 1 day of storage in 22°C in „pepsi" (G1) and in orange juice (G2), 7 days of storage in 22°C in „pepsi" (G3) and in orange juice (G4) and in coffee (G5), 7 days in 0.9% NaCl solution in 60°C (G6), in milk 60°C (G7), in 0.9% NaCl solution in 40°C (G8), in milk 40°C (G9). The results were compared with reference sample.

Results and Discussion

The values of contact angle of acrylic material surface with deposits arising after storage were obtained (FIG. 1 and FIG. 2). The average values of angle were used to determine the SFE and its components, as well as the cohesive energy and solubility parameter (TABLE 1).

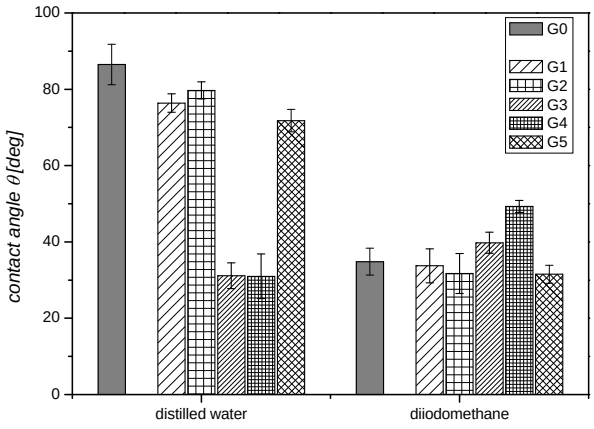

FIG. 1. The contact angle values for groups G1-G5 (room temperature of storage).

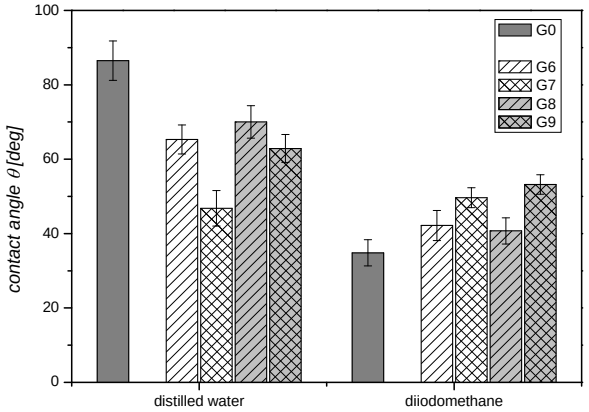

FIG. 2. The contact angle values for groups G6-G9 (temperature of storage 40 and 60°C).

TABLE 1. The comparison of surface properties.

No	γ_S	γ_S^d	γ_S^p	e_c	δ
	[mJm^{-2}]			[MJm^3]	[$mJ^{1/2}m^{-3/2}$]
G0	43.5 (2.1)	42.1 (1.3)	1.4 (0.8)	442.9 (31.5)	21.0 (0.7)
G1	46.7 (1.9)	42.5 (1.6)	4.1 (0.3)	491.6 (31.5)	22.2 (0.7)
G2	46.3 (2.1)	43.4 (1.9)	2.9 (0.2)	485.7 (32.5)	22.0 (0.7)
G3	68.3 (1.8)	40.1 (0.7)	28.2 (0.9)	870.3 (31.7)	29.5 (0.5)
G4	66.1 (2.7)	34.7 (0.7)	31.4 (2.0)	827.7 (50.7)	28.8 (0.9)
G5	49.2 (1.6)	43.5 (0.8)	5.7 (0.8)	532.2 (25.7)	23.1 (0.6)
G6	48.5 (2.7)	38.5 (1.7)	10.0 (1.0)	521.7 (44.5)	22.8 (1.0)
G7	57.0 (3.0)	34.5 (1.2)	22.5 (1.8)	662.8 (51.6)	25.7 (1.0)
G8	46.8 (2.7)	39.5 (1.5)	7.6 (1.2)	493.6 (42.3)	22.1 (0.9)
G9	46.1 (2.5)	32.5 (1.2)	13.7 (1.3)	483.3 (38.8)	21.9 (0.9)

Analyzing the change of the surface energy and its components after the storage, the effect of the fluid nature on the sediment formed on the surface of the material can be clearly seen. The increase of a polar component in each of the tested group, including the largest - 20 times over 7 days of storage in orange juice (G3) and "pepsi" (G4) was observed. The changes of a dispersive component had a different trend, the greatest increase was approx. 4% in the G5 and the decrease was ca. 24% in the G9. The highest value of e_C was shown for G3 and G4.

Conclusions

The mechanisms inducing bacterial adhesion to dental material depends on many factors associated with a substrate, including SFE [2]. Some of consumed liquids influence on substantial increase of SFE value.

References

[1] Pero A.C., et. al, Surface properties and color stability of an acrylic resin combined with an antimicrobial polymer, Revista De Odontologia Da Unesp, 2013, July-Aug; 42(4): 237-242.

[2] Pogorzelski S.J., et. al, A novel methodology based on contact angle hysteresis approach for surface changes monitoring in model PMMA-Corega Tabs system, Applied Surface Science, Volume 258, Issue 8, 1 February 2012, Pages 3652–3658.

[3] Silva W.J., et. al, Influence of surface free energy of denture base and liner materials on Candida albicans biofilms, Journal of Investigative and Clinical Dentistry (2015), 6, 141–146.

[4] Sobolewska E., Frączak B., Błażewicz S. i inni: Porównanie kąta zwilżalności podstawowych materiałów protetycznych stosowanych w wykonawstwie protez ruchomych w badaniach in vitro, Protetyka Stomatologiczna, LIX, 6, 2009.

CONTACT ANGLE AND SURFACE FREE ENERGY OF FRESH AND STORED PIG'S SKIN

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[ENGINEERING OF BIOMATERIALS 143 (2017) 10]

Introduction

Influence of storage conditions of pig's skin tissue properties is important information from the point of view of clinical research, in which a pig's skin is used as a substitute for the human skin. Completing the material for experimentation more than once requires some amount of time to form an adequate research group. Thus, ways of skin sample storage and storage conditions, i.e. time, media (preservation liquids) and temperature are searched for [1,2]. The aim of the research was an evaluation of contact angle and free surface energy for pig's skin stored in different conditions.

Materials and Methods

Skin taken from the back of a 5-month old domestic pig was used in this investigation. Two variants of sample storing were applied before conducting the test: in the isotonic salt solution (0.9%) at the temperature of 4 [°C], and frozen at the temperature of -18 [°C]. The period of sample storing was 24 hours, 5 and 8 days.
The contact angle values were measured with the use of the sessile drop method with the See System computer-based instrument produced by Advex Instruments. Three liquids were used: distilled water (Poch S.A.), diiodomethane (Merck Sp. z o.o.) and anhydrous glycerol (Chempur). The volume of the drop was 0.5 [µl] and the temperature of the test was 22 [°C]. Measurements were carried out at least ten times for each surface. The values of contact angle were shown as the average values with a standard deviation. The obtained values of water and diiodomethane contact angle were used for the skin surface free energy (SFE) according to Owens-Wendt model calculation. The critical surface tension (γ_c) was determined by Zisman plot.

Results and Discussion

The shape of three liquid drops deposited on the surface of fresh and stored skin samples were shown in FIG. 1. The decrease of drops' height after storing in salt solution and freezing can be seen.

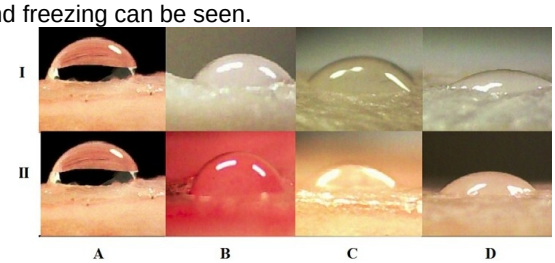

FIG. 1. The shape of drops deposited on skin samples incubated in salt solution (I) and frozen (II): A – fresh sample, B – after 24 hour storage, C – after 5 days storage, D – after 8 days storage

In FIG. 2, the values of contact angle for pig's skin were shown. The storing conditions resulted in decrease of the contact angle values. The change of contact angle values was similar for immersed and frozen samples after 24 hours and 5 days. But after 8 days of storing, significant decrease of the contact angle value can be seen for samples immersed in salt solution.

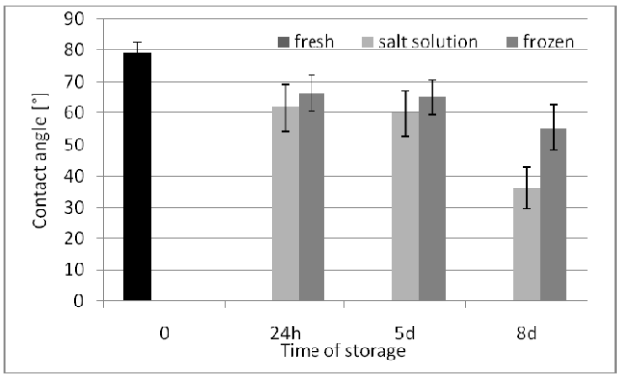

FIG. 2. Water contact angle for fresh and stored pig's skin.

TABLE 1. SFE (γ_s) and its polar (γ_s^p) and dispersive (γ_s^d) components for fresh and stored pig's skin.

	γ_s[mJ/m^2]	γ_s^p[mJ/m^2]	γ_s^d[mJ/m^2]
fresh	43.47	3.71	39.76
Salt – 24h	43.87	16.95	26.92
Salt – 5 days	50.27	13.66	36.61
Salt – 8days	60.72	34.08	26.64
Frozen – 24h	50.77	8.34	42.43
Frozen – 5 days	49.91	9.64	40.27
Frozen – 8 days	49.31	18.58	30.73

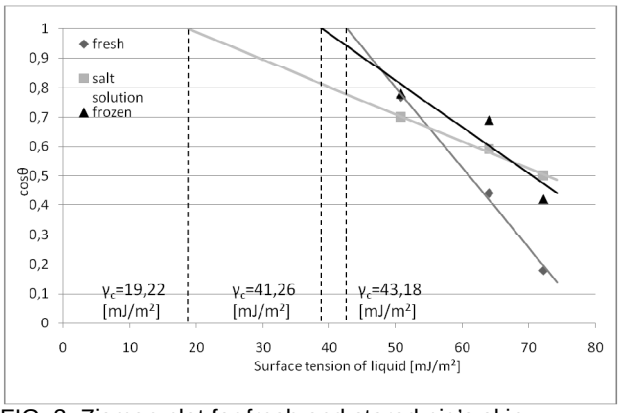

FIG. 3. Zisman plot for fresh and stored pig's skin.

SFE values increased under the influence of storage (TABLE 1). The changes of SFE resulted mainly from the increase of the polar component. The determinated value of the critical surface tension decreased after five days of storage, especially in the case of samples incubated in salt solution (FIG. 3). The obtained values of the water and diiodomethane contact angle and SFE for fresh pig's skin are in good agreement with these values obtained by Krawczyk [3].

Conclusions

Problem of soft tissue samples preservation is key factor in keeping the cellular component of the samples viable. The results showed that measurements of contact angle and SFE values can be one of the method to characterize usefulness of skin samples to mechanical tests.

References

[1] M. Geerligs, Skin layer mechanics, Ph.D. Thesis, Technische Univeriteit Eidhoven, 2010, p.27-30.
[2] P. Elsner, E. Barardesca, Bioengineering of skin. Methods and instrumentation, Boca Raton: CPC Press, 2002.
[3] Krawczyk J., Surface free energy of the human skin and its critical surface tension of wetting in the skin/surfactant aqueous solution/air system, Skin Research and Technology, 2015, 21: 214-223.

EFFECT OF *IN VIVO* BIODEGRADATION ON THE STRUCTURE OF BNC CARDIAC IMPLANTS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 11]

Introduction

Bacterial nanocellulose (BNC), a natural polysaccharide nanomaterial, is synthesized by bacteria of the *Gluconacetobacter* genus, i.a., by *Gluconacetobacter xylinus* strains. In comparison with plant cellulose, contaminated with hemicelluloses and lignines, BNC is characterized by high purity, high degree of crystallinity and polymerization, and good mechanical properties. This material meets the requirements of biomaterials: is biocompatible, not mutagenic, not toxic and not teratogenic. Furthermore, it does not induce either immune responses or tendency to thrombus formation. 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. Although BNC material is not biodegradable in vitro condition, its biodegradation in vivo conditions has not been tested yet. Hence, before using BNC implants in human body it is necessary to carry out pre-clinical tests on animal model.

Materials and Methods

BNC, obtained according to the method described in patents PL 171952 and PL 212003, was supplied by Bowil Biotech Sp. z o.o. The aortic patches implants based on BNC were implanted to pigs body. After 6 months, euthanasia of animals was performed and changes in the structural and morphological properties of the implants were tested by using XRD and SEM techniques, respectively.

Results and Discussion

Presence of the BNC cardiac implant in a pig model body for 6-months, resulted in a change in polysaccharide structure and surface morphology. Results obtained revealed that the crystallinity degree of BNC was deceased compared to the unimplanted sample. In addition, differences in individual cases were noted, possibly related to the biological variability of the examined animals. Microscopic observations showed that natural fiber network structure of BNC was overgrown by biological tissues.

Conclusions

BNC implants integrate with surrounding tissues, so they are biocompatible. After 6 months implantation, they do not biodegrade in the pig animal model.

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”.

References

[1] R. Chandra, R. Rustigi. Prog. Mater. Sci. 23 (1998), 1273 - 1335.
[2] S. Bielecki, H. Kalinowska. Post. Mikro. 47(2008), 163-196.
[3] E. Galwas, A. Krystynowicz. PL 171952 B1(1993)
[4] A. Krystynowicz, W. Czaja, S. Bielecki. PL 212003 B1 (2003)

USE OF CALCITE IN TRICALCIUM PHOSPHATE BASED CHEMICALLY BONDED BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 12]

Introduction

Biomaterials based on calcium phosphates are effective bone substitutes, mainly due to their chemical and mineralogical similarity to the inorganic component of bone. Recently, calcium phosphate bone cements (CPCs), especially on the basis of α-tricalcium phosphate (α-TCP), have been intensively studied [1]. CPCs are characterized by excellent biocompatibility and surgical handiness. Interesting modifier of CPCs is calcium carbonate ($CaCO_3$). Calcium carbonate has three anhydrous polymorphs vaterite, aragonite and calcite [2]. Calcite is a geologically abundant material, which can be used to produce scaffolds for clinical dental and orthopaedic applications It has been proven that $CaCO_3$ can be apply as one of the constituents of CPCs, in order to support formation of carbonated apatite as the end-product of setting reaction. Moreover, presence of calcium carbonate can improve the degradability of the apatitic calcium phosphate cements [3].

Materials and Methods

This study aimed to examine the effect of calcite incorporation into α-tricalcium phosphate based biomaterials. Materials containing 30wt% and 50wt% of calcite were examined. The influence of initial composition on setting times (Gilmore Needles), crystalline phase content (X-Ray Diffraction) and microstructure (SEM) of materials was investigated.

Results and Discussion

Setting times of cements ranged from 11 to 14 min (initial) and from 19 to 27 min (final). The setting depended on the composition of powder and liquid phase. Results of XRD analysis revealed that only two crystalline phases i.e. tricalcium phosphate and calcite were present after 7 days of hardening. SEM observations of fractured cement samples showed that calcite grains were embedded in calcium phosphate matrix (FIG. 1).

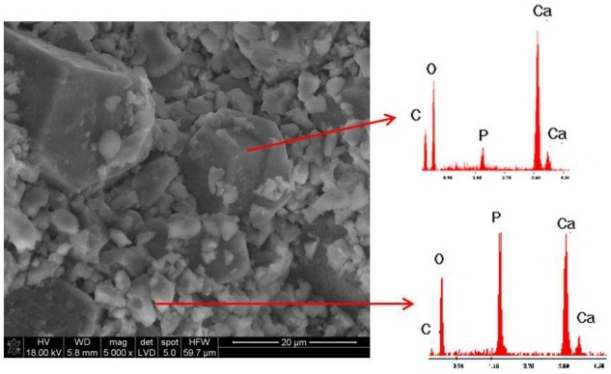

FIG. 1. SEM microphotograph and EDS analysis of fractured cement sample.

Compressive strengths of cements varied from 1.6 to 4.8 MPa. Increasing the amount of $CaCO_3$ decreases the mechanical strength of investigated biomaterials.

Conclusions

Presence of calcite in tricalcium phosphate based cements slightly increases their setting times and decreases the mechanical strength of final materials. Further *in vitro* and *in vivo* studies are required for the complete evaluation of the biomaterials.

Acknowledgments

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References

[1] Czechowska, J., Zima, A., Paszkiewicz, Z., Lis, J., Ślósarczyk, A. Physicochemical properties and biomimetic behaviour of α-TCP-chitosan based materials. Ceramics International, 2014, 40.4: 5523-5532.
[2] Tas, AC. Porous, Biphasic $CaCO_3$-Calcium Phosphate Biomedical Cement Scaffolds from Calcite ($CaCO_3$) Powder. International Journal of Applied Ceramic Technology, 2007, 4.2: 152-163.
[3] Sarıibrahimoğlu, K., Leeuwenburgh, S. C., Wolke, J. G., Yubao, L., Jansen, J. A. Effect of calcium carbonate on hardening, physicochemical properties and in vitro degradation of injectable calcium phosphate cements. Journal of Biomedical Material Research A, 2012, 100A. 3:712-719.

PROGNOSTIC EFFICIENCY OF INFLAMMATORY COMPLICATIONS DEVELOPMENT OF DENTAL IMPLANTATION BASED ON INDICATORS OF LIPID PEROXIDATION (LPO) OF ORAL FLUID

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[ENGINEERING OF BIOMATERIALS 143 (2017) 13]

Abstract
The frequency of complications of dental implantation at the present stage varies from 6% to 23%, what determined the relevance of this examination.
The aim of this work is to determine the predictive effectiveness of the development of inflammatory complications of dental implantation on the basis of indicators of lipid peroxidation (POL) of the oral fluid (level of malondialdehyde (MDA) and the activity level of superoxide dismutase (SOD)).
We examined 49 patients 25-59 years old which had no traumas, operations and diseases requiring medical rehabilitation, inflammatory disease in the maxillofacial area and the gastrointestinal tract, as well as other factors that can affect qualitative and quantitative content of the oral fluid. These patients underwent delayed dental implantation (one implant in one segment of the jaw). The POL indices were determined twice: before operation of dental implantation and 3 days after operation. The effectiveness of the proposed method was determined in accordance to the clinical and economic studies requirements.
Prognostic efficiency in accordance with the procedure of conducting clinical and economic examinations on the basis of MDA level was 78%, and based on the level of SOD activity – 76%, which classifies it as high and allow to reduce the number of complications and diagnostic mistakes including in the planning and execution of invasive procedures associated with the installation of dental implants.

Introduction
The frequency of complications of dental implantation at the present stage varies from 6% to 23% [1]. At the same time laboratory methods of examination because of the subjectivity and the lack of informativeness does not allow to use them to predict these complications in the preclinical stage of development. In the literature there are reports emphasizing the need to develop screening criteria activity and severity of inflammatory-destructive processes in dental implantation on the basis of use for the study of non-invasive biological environment, the oral fluid farst of all [3]. There are some reports on the methods of prognostication of development of peri-implantitis on the basis of biochemical indicators of oral fluid [4], what determined the relevance of this study.

Materials and Methods
The study included 49 patients 25-59 years old which had no traumas, operations and diseases requiring medical rehabilitation, inflammatory disease in the maxillofacial area and the gastrointestinal tract, as well as other factors that can affect qualitative and quantitative content of oral fluid. These patients underwent delayed dental implantation (one implant in one segment of the jaw). A method of predicting of inflammatory complications development of dental implantation based on the level of malondialdehyde (MDA) level and superoxide dismutase activity (SOD) in oral fluid was used in each patient twice: 1) before the operation of dental implantation, 2) 3-days after the operation. Efficiency of the offered method was determined in accordance with the procedure of conducting clinical and economic studies [2].

Results and Discussion
On the 3-rd day after surgery in 38 (77.6%) patients were defined normal indices of MDA level and 40 (81.6%), indicating no risk of inflammatory complications. In 11 (22.4%) of patients were diagnosed the risk of developing these complications on the level of MDA and 12 (24.5%) − the level of SOD activity. Treatment plan of these patients was subjected to correction with the use of surgical and therapeutic methods in order to prevent the development of complications or further development of the pathological process and to avoid rejection/removal of the implant. In long-term follow up 45 patients (91.8%) had prosthetic operation and were satisfied by immediate and long-term results of treatment and rehabilitation. Number rejection/removal implants due to the development of peri-implantitis was 4 (8.2%). Prognostic efficiency in accordance with the procedure of conducting clinical and economic studies on the basis of MDA level was 78%, and based on the level of SOD activity – 76%, which classifies it as high.

Conclusions
The application of prognostication the development of inflammatory complications of dental implantation on the basis of MDA level and the activity level of SOD oral liquid will reduce the number of complications and diagnostic mistakes including in the planning and execution of invasive procedures involved in installing dental implants and also in assessing both immediate and long terms results of treatment.

References
[1] Aziz S.R. Hard and soft tissue surgical complications in dental implantology, Oral Maxillofac. Surg. Clin. North. Am.: 2 (14), 313-318, 2015.
[2] Grakovich A.A. The procedure for conducting clinical and economic studies: application data sheet, Minsk: 34, 2008.
[3] Mashchenko I.S. Risk factors and predict the development of inflammatory complications and local secondary osteoporosis in the bone structures of the jaws in dental endosteal implantation in healthy patients, Klinichna medicine: 1 (18), 19-27, 2012.
[4] Pohodenko-Chudakova I.O., Shevela T.L. A method for predicting the risk of periimplantitis in dental implantation, Afryki bull. Vinahost, carinya model, promyslovye try: 1, 120-121, 2016.

COMPARATIVE APPRAISAL OF OSTEOPLASTIC RESOLVED MEMBRANES FOR PREVENTION OF THE ALVEOLAR PART LOWER JAWS ATROPHY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 14]

Introduction
One of the urgent problems in oral and maxillofacial surgery is prevention of alveolar bone atrophy [1,2]. This problem determines: the significant spread of tumours and tumour-like bone diseases, alveolar ridge decreases after typical and atypical tooth extraction operations [3,4]. The aim of the trials − to study comparatively osteoplastic bioresorbable membranes "Collapan" and "Collost" used for the prevention of the mandible alveolar part atrophy.

Materials and Methods
42 individuals (men, 45 to 70 years) were involved in trial. The first group included 20 individuals in whom we used "Collapan" in surgical treatment. This was a comparison group. Group 2 included 22 patients in whom we used the "Collost" membrane. The individuals were undergone oral surgery of atypical tooth extraction, cystectomy with apex root resection made by standard operative protocols with standard anti-inflammatory therapy. Clinical efficacy was assessed on the number of complications in the postoperative period. The level of the alveolar bone atrophy was assessed at the long-term follow-up (after 1 year) on the parameters of the mandibular bone tissue determined on the radiological data (cone-beam computed tomography).

Results and Discussion
In the postoperative period in group 1 there were revealed 6 (14%) facts of infectious-inflammatory nature complications - alveolitis. 2 (5%), in group 2 - 2 (5%) complications: 1 (2.5%) – alveolitis, 1 (2.5%) – forced tooth extraction.
The results of clinical radiographic evaluation of the bone wounds healing in the comparison groups after 1 year allowed us to come to the conclusion that using of barrier membranes in oral surgery in general, optimizes the pace of reparation and regeneration due to osteoinductive and osteoconductive properties of these materials and contributes to the prevention of alveolar atrophy. “Collost” demonstrated more pronounced effectiveness according obtained data in comparison with the membrane “Collapan”.

Conclusions
The osteoplastic bioresorbable membrane "Collost" is mostly appropriate for the prevention of the lower jaw alveolar part atrophy.

Acknowledgments
To Belarusian State Medical University.

References
[1] Guided bone regeneration by means of a preformed titanium foil: A case of severe atrophy of edentulous posterior mandible / B. M. Andreasi [et al.] J. Biol. Regul. Homeost. Agents. – 2016. – Vol. 30, Suppl 1. – P. 35−41.
[2] Krasny M., Krasny K., Fiedor P., Zadurska M., Kamiński A. Long-term outcomes of the use of allogeneic, radiation-sterilised bone blocks in reconstruction of the atrophied alveolar ridge in the maxilla and mandible, Cell Tissue Bank: 16 (4), 631−638, 2015.
[3] Krasilnikov A.A., Nikolaev S.M., Markaryan A.A., Mondodoev A.G., Gulyaev S.M. Influence of Collost and Collost with chymotrypsin on bone defect healing, Izvestiya Dagistani Ped. University. Natural and Exact Sci.: 4, 75-79, 2011.
[4] Lima-Verde-Osterne R., Turatti E., Cordeiro-Teixeira R., Barroso-Cavalcante R. The relative frequency of odontogenic tumors: a study of 376 cases in a Brazilian population, Med. Oral Patol. Oral Cir. Bucal.: 22 (2), e193−e200, 2017.

TREATMENT OF ACUTE PERIOSTITIS OF JAWS WITH THE BACTERIOPHAG

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[ENGINEERING OF BIOMATERIALS 143 (2017) 15]

Introduction
Acute odontogenic periostitis of the jaws is one of the most common inflammatory diseases in the outpatient surgical dentistry. During the pus research mixed microflora is revealed in the case of acute purulent periostitis: various Streptococci and Staphylococci species, Gram-positive and Gram-negative rods, putrescent bacteria. 75% of them are anaerobic and 25% refers to aerobic flora [1].
The aim of the trials − to study the possibility of using the drug «Sextaphag» (pyobacteriophag polyvalent) in the treatment of acute odontogenic periostitis of jaws.

Materials and Methods
The trials were carried in “5-th Minsk city outpatient clinic” in 10 patients of 26 - 53 years with acute odontogenic periostitis of jaws. 2 patients had comorbidities: one patient had thyroid gland disease (nodular goiter) and one patient was 16 weeks pregnant. After the primary surgical treatment of purulent focus it was injected 1.0 ml of «Sextaphag» solution once in periostotomical wound. Drug treatment was according the clinical protocol. The efficiency was estimated according the clinical picture.

Results and Discussion
On the first day after operation we observed in patients: the body temperature 37.2-37.6°C, asymmetric face with collateral soft tissues swelling, enlarged submandibular lymph nodes 1.5−2.0 cm in diameter. There were revealed edematose, hyperemic, painful oral cavity mucosa in periostoetomical wound area, profuse, purulent exudation on drainage. On the second day there were revealed reduction of: lymph nodes size and there painfulness, of mucosal swelling around periostoetomical wound, of exudation from the periostotomical wound. There were no clinical symptoms on the third day of patients careering.
The adverse reactions were not observed during applying the «Sextaphag» drug, also in pregnant women and patient with nodular goiter.
The drug “Sextaphag” consists of a sterile filtrate of fagolisated bacteria Streptococcus, Staphylococcus, Proteus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli enteropathogenic strains, so it has the specifically lysing ability of them and affects entire microorganisms’ spectrum that cause odontogenic periostitis of the jaws.

Conclusions
The drug «Sekstafag» can be used successfully in acute odontogenic periostitis of jaws local treatment.

Acknowledgments
To Belarusian State Medical University.

References
[1] Kabanova, A.A. Oral and facial surgery and surgical dentistry: a pocket book of a doctor / A.A. Kabanova - Medical literature, 2015. - 160 p.

BIOMATERIAL MODIFICATIONS AND CELLULAR BEHAVIOR

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[ENGINEERING OF BIOMATERIALS 143 (2017) 16]

Introduction

The first critical course for assessing the suitability of a new biomaterial in medicine is - in addition to the biofunctionality - the compatibility of the biosystem at the site of its effect. Knowledge of the molecular interactions of biomaterial surface modifications – topography and chemistry - with the biosystem is of specific importance. Osteoblasts, important cell type in orthopedic and dental applications, are known to sense the biomaterial surface characteristics [1]. The most central initial process in this cell-material interaction is the mechanical anchoring of the cell to the biomaterial interface – the cellular adhesion (FIG. 1) [2]. The "race for the surface" is decisive for the integration and acceptance of biomaterials [3]. The contacts via adhesion molecules like the integrins lead to modulated cell functions such as signaling events [4]. External signals from physico-chemical environments finally affect the cell physiology [5,6]. Therefore, it is important to study *in vitro* effects of biomaterials on cellular adhesion, the expression and location of the actin cytoskeleton, the expression of signaling molecules and finally cell function markers [5,6]. The understanding and interpretation of the cellular behavior via biophysical *in vitro*-studies is critical for the acceptance of new biomaterial surfaces in medicine.

Materials and Methods

In our field of research, we conducted the *in vitro*-studies on the acceptance and functionality of newly developed biomaterials. In addition to biomaterial tests, we also specifically investigated the influence of a defined topography or chemistry to provide insights in cellular behavior [5,6]. To analyze the cell morphology we used microscopy – scanning electron microscopy (FE-SEM) or confocal laser scanning microscopy (LSM). The flow cytometry was applied for the expression of cellular components or cell cycle regulation. To determine further specific cellular markers and reactions, many techniques are used such as rtPCR, Western-Blot, ELISA or Bio-Plex. The biological characteristics of a range of materials (titanium, silicon, polymers or ceramics) are studied *in vitro* with relevant osteoblast-like cells as well as primary cells in the appropriate media under physiological conditions: 37°C, 5% CO_2 [4-6]. To validate the data we used GraphPad Prism with the corresponding test of significance.

Results and Discussion

Established on our basic research, we were able to demonstrate that a defined surface topography influences the osteoblast behavior: changes in cell morphology, the organization and expression of cellular structures, signaling events and finally also the expression of bone-specific markers. Morphological analyses with FE-SEM as an important parameter revealed the cellular behaviour on biomaterials (FIG. 2). Using LSM we were able to recognize the influence of nano- and microstructures and chemical modifications on the spatial organization of cellular components, e.g. the actin cytoskeleton (FIG. 3). These studies of the cell architecture and physiology are first important steps for assessing cellular behavior at the interface of a biomaterial.

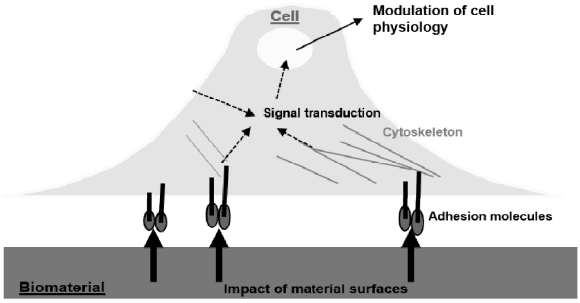

FIG. 1. Scheme of cell-material interaction.

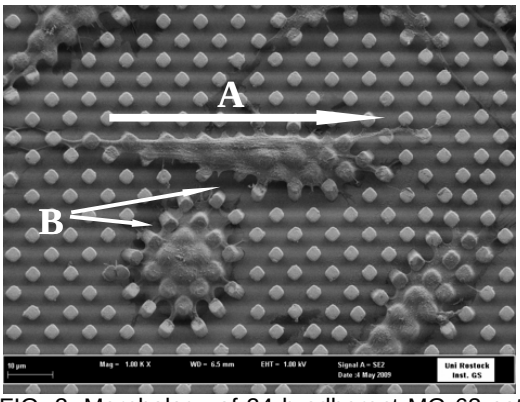

FIG. 2. Morphology of 24 h adherent MG-63 osteoblasts on defined micro pillars (Si-Ti, 3x3x5 µm, W x L x H by RIE; Prof. Kern/Tubingen) (FE-SEM, magnification 1kx, bar 10 µm, Lange/Rostock). Note that cells are elongated and oriented along the structures (A). The cells are able to pull the pillars due to their adhesion strength (B).

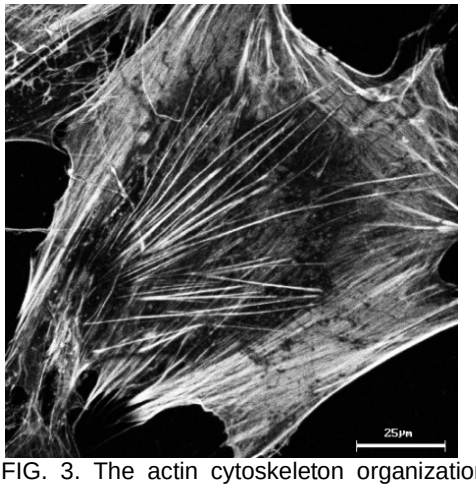

FIG. 3. The actin cytoskeleton organization of primary osteoblasts on the planar surface Si-Ti (LSM410, bar 25 µm). Note the well-defined stress fibers spanning across the entire cell body.

Conclusions

Cell biologic *in vitro*-studies are necessary for a better understanding and assessment of innovative medical materials and their interplay with the surrounding biosystem.

Acknowledgments

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References

[1] J. Rychly & J.B. Nebe, BioNanoMat 14 (2013) 153-60.
[2] K. Anselme, Biomaterials 21 (2000) 667-81.
[3] A. Gristina, Science 237 (1987) 1588-95.
[4] F. Luethen *et al.,* Biomaterials 26 (2005) 2423-40.
[5] C. Moerke *et al.,* Biomaterials 76 (2016) 102-14.
[6] S. Staehlke *et al.,* Biomaterials 46 (2015) 48-57.

SHORT CALCIUM PHOSPHATE WHISKERS FOR MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 17]

Introduction

Synthetic hydroxyapatite (HA) has been successfully used for biomedical (especially orthopedic) applications, because of its close compositionally resemblance to the natural bone [1-3]. HA is one of the components of composites used in bone regeneration very often. The composites usually require an appropriate durability, so most of the researches have focused on the use of HA whiskers as promising reinforcement for such materials [4]. It was also found that the HA whiskers are non-toxic and are compatible with human body. The application of well-crystalized, stoichiometric HA whiskers in resorbable composites for bone regeneration may be limited due to its slow resorption [5], so more appropriable seems to be using resorbable calcium phosphates or whiskers being bi- or triphasic mixtures.
There are a number of different methods for producing calcium phosphate whiskers [6-8]. The most frequently hydrothermal homogenous precipitation is used. But hydrothermal synthesis procedure requires special autoclaves enabling heating of aqueous solutions to high temperatures up to 200°C [9.10].
The present work shows the results of synthesis of calcium phosphate whiskers through the method described in literature [11], by the hydrolysis reaction of β-tri-calcium phosphate (β-TCP) in presence of H_2O_2. The process was carried out in considerable lower temperature than for hydrothermal methods. The formation, morphology and phase composition of whiskers obtained were studied as a function of temperature and time of reaction.

Materials and Methods

Calcium-phosphate whiskers were synthesized in one-pot technique. 4 grams of β-TCP powder (Fluka) were placed in 250 ml capacity Pyrex glass bottle. Then, 100 ml of 30% solution of H_2O_2 (Avantor) was added. The bottle was capped and shaken for 2 min, followed by heating for 8-96 hours in an electric dryer in 90-95°C. The final whiskers from the bottle were filtered, washed with distilled water and dried overnight at 90°C.
The morphology and phase composition of whiskers were analyzed by scanning electron microscopy (SEM) and X-ray diffractometry (XRD), respectively. Functional groups of the samples were identified by Fourier transform infrared spectroscopy (FTIR).

Results and Discussion

Test results indicate that using the above mentioned procedure calcium phosphate whiskers were obtained. As can be seen at FIG. 1, the synthesis led to form whiskers and aggregates with different sizes (from several to tens μm). The morphology and size of these whiskers depend on time and temperature of the reaction. The length and width of whiskers increase with increasing of time and temperature.

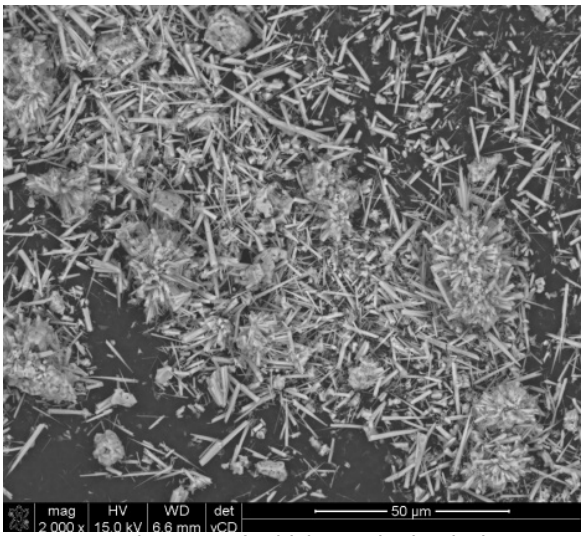

FIG. 1. SEM images of whiskers obtained via one-pot technique.

Phase analysis of the obtained product remains with opposite to the literature's data [11] where produced whiskers were found to be biphasic mixtures of apatitic CaP and octacalcium phosphate ($Ca_8H_2(PO_4)_6{\cdot}5H_2O$). In our tests XRD patterns revealed the triphasic mixture of: hydroxyapatite, calcium pyrophosphate ($Ca_2P_2O_7$) and residue of β-tri-calcium phosphate. These results are confirmed by FTIR spectra. The amount of β-tri-calcium phosphate decreased with the time of reaction up to 15 wt%, the amount of hydroxyapatite increased up to 72 wt%, whereas the content of calcium pyrophosphate is almost stable and decreased only insignificantly from 16 to 12 wt%.

Conclusions

Applying one-pot technique of synthesis using β-TCP powder and 30% solution of H_2O_2, calcium phosphate whiskers were obtained. They were characterized as a triphasic mixture of HA, calcium pyrophosphate and β-TCP.
Test results indicate that there are at least two factors (temperature and time of reaction) that simultaneously affect the morphology and size of the obtained whiskers.
Such whiskers may be useful as promising reinforcing filler for composite of increased mechanical strength.

Acknowledgments

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References

[1] S.M. Best, A.E. Porter, E.S. Thian, J. Huang, J. Eur. Ceram. Soc. 28 (2008) 1319-1327.
[2] H. Zhang, Y. Wang, Y. Yan, S. Li, Ceram. Int. 29 (4) (2003) 413-418.
[3] S. Jalota, S.B. Bhaduri, A.C. Tas, J. Biomed. Mater. Res. A 78 (3) (2006) 481-490.
[4] F. Zhou, F. Qingling, Mat. Sci. Eng. C 35 (2014) 190-194.
[5] M. Bohner, U. Gbureck, J.E. Barralet, Biomaterials 26 (2005) 6423-6429.
[6] R.K. Roeder, G.L. Converse, H. Leng, W. Yue, J. Am. Ceram. Soc. 89 (7) (2006) 2096-2104.
[7] A.C. Tas, J. Am. Ceram. Soc. 90 (8) (2007) 2358-2362
[8] H. Zhang, B.W. Darvell, J. Eur. Ceram. Soc. 30 (10) (2010) 2041-2048.
[9] J.G. Li, T. Hashida, J. Am. Ceram. Soc., 89 (2006) 3544-3546.
[10] Y. Mizutani, M. Hattori, M. Okuyama, T. Kasuga, M. Nogami, J. Eur. Ceram. Soc., 25 (2005) 3181-3185.
[11] A.C. Tas, J. Am. Ceram. Soc. 90 (8) (2007) 2358-2362.

MICROSTRUCTURE OF CHITOSAN-BASED, ZnO-DOPED ANTIBACTERIAL COMPOSITE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 18]

Introduction

Biomaterials for regenerative medicine, are subjected to continuous improvement, by i.e. amending their integration with body tissues or giving them bactericidal properties [1,2]. The porosity and sizes of pores enables appropriate migration of cells and ensures easy delivery of nutrients within the implant, appear to be of significant importance [3]. Literature reports suggest that natural and synthetic, biostable and biodegradable polymers as well as calcium phosphates (e.g. hydroxyapatite, TCP) or Hench's bioglass [4], which have no antibacterial properties, are currently used in tissue engineering. Chitosan is also widely used in medicine. Its main characteristics include biocompatibility, antimicrobial and hemostatic properties, and biodegradability [5]. Due to the osteoconductive properties it is suitable for hard tissue engineering [6]. Silver is most commonly used as an antibacterial agent, but other metal ions (i.e. Zn^{2+}) are also characterized by bactericidal properties [7].
The purpose of the study was to determine the effect of glass composition, amount of polymer used and preparation method on microstructure of chitosan-based antibacterial composites, suitable for use in regenerative medicine.

Materials and Methods

Porous biocomposites based on chitosan solution and 1wt% or 2wt% ZnO doped bioglass from $CaO\text{-}SiO_2\text{-}P_2O_5$ system were fabricated using lyophilization method. Composite microstructure was controlled by the appropriately selected amount of bioglass in relation to the polymer, its appropriate grain size and by the amount of the solvent present in lyophilizated dispersions.
The morphologies of the resulting composites were determined by scanning microscope observations. Their bioactivity was assessed by comparing SEM-EDS analysis of chemical compositions before and after contact with SBF solution. By measuring the specific surface area using BET method, the effect of the dispersion composition on development of the surface of composites was determined. The evaluation of cytotoxicity was performed according to PN-EN 10993-5 "Biological evaluation of medical devices - Part 5: Tests for cytotoxicity: *in vitro* methods" after contact with fibroblast like cells L929. Studies of antimicrobial activity were performed by a dilution method, using precultures of test bacterial strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Results and Discussion

All obtained composites showed random distribution of bioglass grains in polymer matrix with no visible agglomerates. The cross-section SEM image of the P5Zn1gCH_1:1 composite (FIG. 1) shows the structure of internally connected open pores of sizes in range of 40–300 μm. The increase of bioglass amount in relation to polymer results in a more distorted microstructure of the composite. Also, the presence of bioglass of larger grains disrupts composite microstructure and causes deformation of the pores. The change of solvent amount affects the stability of the dispersions in lyophilization, the shape and the size of composite pores, as well as its rigidity. Specific surface area that determines the kinetics of ion release, which indirectly affects bioactivity, for P5Zn1gCH_1:1 composite was 127.68 ±0.02 m^2/g. Changes in intensity of Si, Ca and P signals on EDS spectra indicate that apatite layer formation starts after composites incubation in SBF solution.

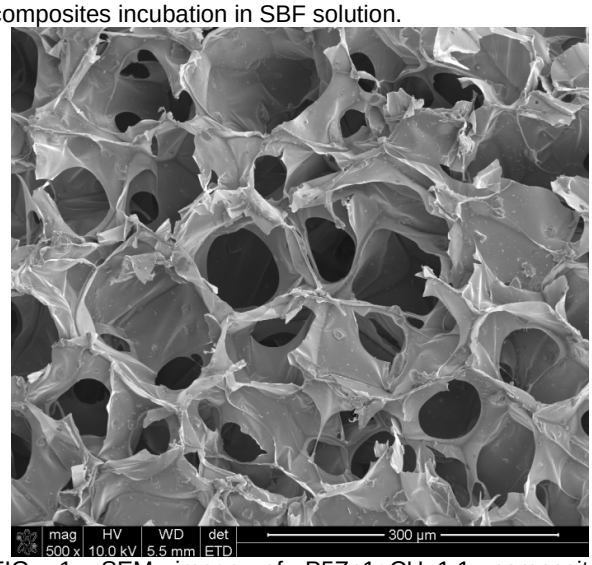

FIG. 1. SEM image of P5Zn1gCH_1:1 composite microstructure.

Morphological images of cell cultivations in the indirect method, after a contact of L929 with eluates of examined composites, were correct, and proliferation indicated after 48 h was tending to increase. Results of antimicrobial activity indicate that all of obtained materials caused the reduction of the number of bacteria. The best results were obtained for P5Zn2CH_1:1 composite doped with 2% of ZnO.

Conclusions

Chitosan-based, ZnO-doped antibacterial biocomposites have an optimal mean pore size for bone tissue ingrowth as well as exhibit antibacterial activity against the strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The composites microstructure could be controlled by changing of the preparation parameters.

Acknowledgments

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References

[1] L. Ciołek, J. Karaś *at al.*, Engineering of Biomaterials, vol. XI, nr 77-80, (2008) 25-27.
[2] H.Zhu, Ch Hu., *at al.*, Mater. Sci. Eng., C, 42 (2014) 22-30.
[3] BS Kim, IK Park *et al*., Prog. Polym. Sci. 36(2011) 238-268.
[4] AM El-Kady, EA Saad *et al*., Ceram. Int.,36(2010) 995-1009.
[5] A. Di Martino, M. Sittinger *et al*., Biomaterials, 26(2005) 5983-5990.
[6] PR. Sivashankari, M. Prabaharan *et al.,* Int. J. Biol. Macromol., B. 93(2016) 1382-1389.
[7] T.N. Kim, Q.L. Feng, *at al.*, J. Mat. Sci – Mater. Med., 9 (1998) 129-134.

STRUCTURE AND MORPHOLOGY OF WHITLOCKITE COATING ELECTROPHORETICALLY DEPOSITED ON NiTi SHAPE MEMORY ALLOY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 19]

Introduction

The NiTi shape memory alloys (SMA) with its chemical composition near to that of equiatomic ones are used in a wide range of biomedical fields. Their biomechanical properties make it more suitable for bone fixation than other metallic materials [1]. In order to improve biocompatibility, the surface of NiTi alloys is modified by producing biocompatible layers [2]. It is desirable that the protective layers increase the functionality of the implant surface, for example by enhance the osseointegration. The best bonding of the metal implant surface to bone tissue is achieved by producing calcium phosphate-based coatings (CaPs) such as hydroxyapatite (HAp) or whitlockite ceramic (TCP) [3].

The main aim of presented results was focused on the biocompatibility intensification of NiTi SMA done by its surface modification. The technology of material preparation was concentrated on the passivation of NiTi substrate by autoclaving and following electrophoretic deposition (EPD) of whitlockite ceramic (β-TCP).

Materials and Methods

A NiTi alloy with the following chemical composition of 50.6 at.%. Ni and 49.4 at.%. Ti (Memry GmbH) was used as substrate for layers deposition. Before EPD, the substrate was passivated in autoclave at 134°C for 30 min. The autoclaving resulted in formation of a thin amorphous TiO_2 layer what improves the corrosion resistance and provides a stable connection of deposited ceramic particles to metal substrate [2,4].

The powder of whitlockite (nGimat) consisted of 87.1 ±1.0 wt% β-$Ca_3(PO_4)_2$ and 12.9 ±0.2wt% β-$Ca_2P_2O_7$ was used to prepare a colloidal suspension having a concentration of 0.1wt% the powder in 99.9% ethanol (Avantor). Next, the mixture was put into a magnetic stirrer (30 min) and then transferred to an ultrasonic bath (30 min). The average size of the particles was ca. 550 nm [5]. Afterwards, electrophoretic deposition (EPD) under cathodic condition and room temperature was performed to cover the NiTi substrate by CaPs particles. The constant voltages (from 20 to 80 V) at time periods (from 30 to 120 s) were applied. After deposition, the green form coatings were dried for 24h at ambient temperature. Then, in order to consolidation and increase the adhesion strength of the ceramic coating to the metal substrate samples were heat treated in vacuum furnace at 1000°C for 2h.

Results and Discussion

The outcomes revealed that applied voltage and deposition time have a great impact on the quality of whitlockite coatings electrophoretically deposited on the passivated NiTi substrate. Due to lower voltage and deposition time, the ceramics particles spread on the surface heterogeneously forming larger agglomerates. Increase of the quantity of deposited particles, at the constant voltage with elongation deposition time, in comparison to constant time and increase of voltage was observed. Deposition parameters such as 80V/120s impact on a significant increase in the density and thickness of the coating. The applied heat treatment conditions (1000°C for 2h) resulted in a visible change in the morphology of the coating. The areas between the agglomerates changed from smooth to rough. It may be caused by the intensification of the titanium oxide crystallization from the amorphous oxide layer, previously formed on NiTi substrate during the autoclaving. The presence of crystallized clusters of fine particles, especially close to CaP aggregates, was also stated (FIG. 1).

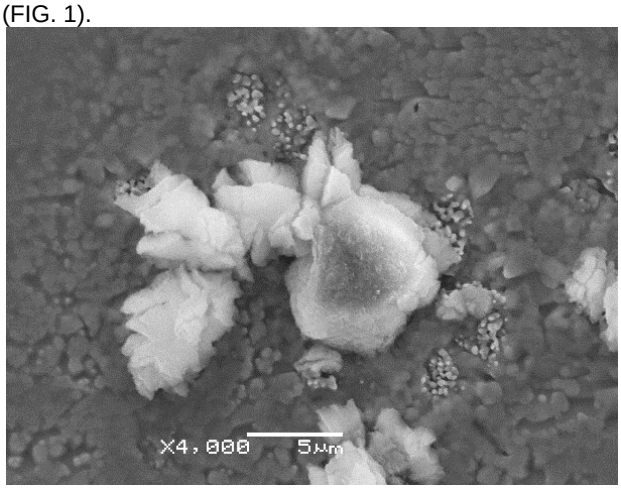

FIG. 1. SEM image of surface observed for the NiTi coated substrate.

Diffraction data (GIXRD) collected for samples after deposition and after sintering process reveled well-defined peaks both of β-TCP (ICDD - PDF 04-008-8714) with rhomboedral structure (R-3c) and trace amount of β-$Ca_2P_2O_7$ phase with tetragonal structure ($P4_1$) (ICDD-PDF 04-009-8733). The crystal structure of coatings materials remain unchanged in comparison to starting material. The presence of diffraction lines from whitlockite decomposition products was not proved. The applied heat treatment (1000°C / 2h in vacuum) resulted in a partial decomposition of NiTi substrate (ICDD - PDF 01-078-4618) to equilibrium phases: Ti_2Ni (ICDD - PDF 04-007-1531) with cubic structure (Fd-3m) and Ni_3Ti with hexagonal structure ($P6_3/mmc$). In addition, the appearance of diffraction lines belonging to non-stoichiometric $TiO_{0,325}$ with hexagonal structure ($P6_3/mmc$) (ICDD - PDF 04-005-4356) and TiO with cubic structure (Fm-3m) (ICDD - PDF 04-016-4319) were be identified.

Conclusions

Application of deposition voltage of 20V for 60s resulted in homogenous covering of passivated NiTi substrate by whitlockite layer. As a result of heat-treatment (1000°C / 2h in vacuum) crystallization of titanium oxides and partial decomposition of NiTi alloy were observed. However, the structure of CaP coating material remains unchanged.

References

[1] H. Morawiec, Z. Lekston: Implanty medyczne z pamięcią kształtu. Wydawnictwo Politechniki Śląskiej, Gliwice (2010).
[2] J. Lelątko, T. Goryczka: Modyfikacja powierzchni stopów NiTi wykazujących pamięć kształtu. Oficyna Wydawnicza WW, Katowice (2013).
[3] S. V. Dorozhkin, Materials 2, (2009), 399-498.
[4] T. Goryczka, K. Dudek, Powder Diffraction (in press) DOI: 10.1017/S0885715617000239.
[5] K. Dudek, M. Plawecki, M. Dulski, J. Kubacki, Mater.Lett.157 (2015) 295-298.

ONE-STEP FABRICATION OF GENTAMICIN NANOPARTICLES EMBEDDED IN POLYMERIC BIOMATERIALS SURFACE: SONOCHEMICAL APPROACH

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[ENGINEERING OF BIOMATERIALS 143 (2017) 20]

Introduction

The most common postoperative complications after implantation are inflammation and infection. A solution to the problem can be controlled drug delivery from the surface of the implanted device which can bring several advantages for patients. Currently, such strategy is one of the most rapidly advancing areas of the biointerface development, offering numerous advantages: in–site treatment, effectiveness, reduced toxicity, and improved patient convenience. The most important benefits are the application of smaller doses of the drug and its delivery directly to the target tissue. Controlled site–specific drug delivery offers an attractive alternative to the typical administration. What is more, such delivery allows the achievement of necessary therapeutic doses at the desired location, while maintaining low or negligible systemic level.

Among several methods, which can be used to fabricate antibacterial surfaces, sonochemistry has proved to be a very effective technique, particularly for polymeric surfaces. The principles are based on the ultrasonic irradiation of water–soluble antibiotic which leads to the formation of nanoparticles (NPs). When the irradiation is performed in the presence of a polymeric surface, the antibiotic NPs are subsequently embedded into the exposed surface in a one–step process. This strategy allows obtaining a composite NPs/polymer with prolonged antibiotic release from the surface.

The aim of this study was to generate gentamicin nanoparticles under ultrasound irradiation, subsequently embed them into oxygen plasma modified parylene C films in a one–step reaction, evaluate the drug surface distribution and resultant elution kinetics.

Materials and Methods

Experiments were performed with chemical vapor deposition (CVD) prepared Parylene C (8 μm of thickness) films, provided by ParaTech Coating Scandinavia AB. To modify the parylene C surface, oxygen plasma treatment was carried out using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany). Gentamicin sulphate nanoparticles were formed and deposited on the oxygen plasma modified parylene C using homogenizer (Sonics Vibracell CV18) with the frequency of 20 kHz, amplitude 30%, and time 6 min. The size of the sonochemically formed GNPs was determined using LM10 Nanosight instrument (Malvern Instruments Ltd) equipped with a sCMOS camera (Hamamatsu Photonics, Hamamatsu, Japan) and a 450 nm blue laser. Data were processed with NTA software version 3.1 Build 3.1.45.

FTIR imaging analyses of the polymeric films were performed in reflectance mode on a Spectrum Spotlight 400 FTIR microscope connected to a Spectrum 100 FTIR spectrometer (PerkinElmer, Inc.). The images were taken at a resolution of 8 cm^{-1} between 4000 and 700 cm^{-1} with 16 scans per pixel.

Drug release studies of the parylene C with sonochemically embedded GNPs were performed in phosphate buffered saline solution (PBS). The prepared samples were placed in 4 mL of PBS (Lonza) and transferred into an orbital shaker−incubator (Biosan, ES-20/60) set at 130 rpm and 37°C.

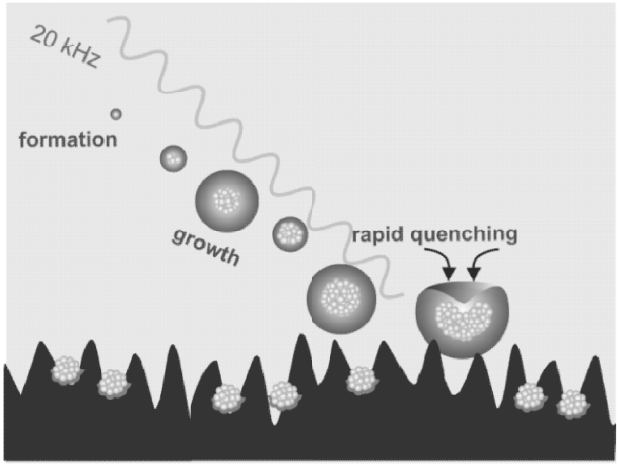

FIG. 1. Schematic representation of the sonochemical synthesis of genaminic nanoparticles and embedding to the nanopoares of parylene C surface.

Results and Discussion

It was found that using sonochemistry, gentamicin nanoparticles in the size range 35-70 nm can be obtained. The presence and homogenous distribution of the drug was confirmed using IR–image technique. The collected spectra revealed a characteristic band at 1037 cm^{-1} for gentamicin (group C–N, C–O). The corresponding absorbance maps (20 μm×20 μm) were collected at this characteristic selected wavelength.

Drug elution studies were performed to determine stability of the gentamicin NPs deposited on the parylene C. The average sample (2 cm^2) drug load was 3 μg. It was found that GNPs/parylene C system provided drug elution time up to 7 days. The obtained data were fitted into the first order kinetic and Korsmeyer–Peppas models. The experimental results were in good agreement only with Korsmeyer–Peppas (R^2 = 0.9796, n = 0.308, and k = 30.77) with the diffusion dominated mechanism. The drug elution is within the therapeutic window (MIC = 2 μg/ml), the GNPs/parylene C system can be successfully used as a coating with therapeutic function preventing contamination of the implant surface before surgery and actively lower the risk of post–operation infection.

Conclusions

It was concluded that sonochemical synthesis gives an effective alternative to biodegradable–based therapeutic layers providing prolonged elution of the active substance to the surrounding tissue which was proved for the GNPs/parylene C system as a representative example.

Acknowledgments

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OXYGEN PLASMA TREATMENT OF PARYLENE C FOR IMPROVED BIOCOMPATIBILITY: MOLECULAR DYNAMICS INSIGHTS INTO WATER-POLYMER INTERACTIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 21]

Introduction

Solid–water interfaces play a vital role in biomaterials science since they provide a natural playground for most biochemical reactions and physiological processes. This is the reason why the functionalization of surfaces is brought to the centre of interest with the aim of optimizing the implant−tissue interface. For the tuning of the properties of medical device surfaces, oxygen plasma is the most commonly applied. An apparent benefit is surface cleanliness; however, the key issue is the incorporation of oxygen-containing surface functional groups such as −COOH, −OH, and −CHO into the originally oxygen free polymers, i.e. protective parylene C (poly(chloro-p-xylylene).

Molecular dynamics (MD) simulations have been employed to investigate with atomistic resolution interactions between water molecules and solid surfaces; however, they are typically focused on model systems such as silica or graphite which are far from polymeric surfaces.

The aim of the study was to perform *in silico* simulations of atomistic-level interactions between water molecules and the polymeric surfaces of parylene C with the idea to compare them with experimental results of surface wettability and osteoblasts adhesion.

Materials and Methods

Fully atomistic molecular dynamics simulations were performed to investigate interactions between water molecules and several surfaces modelling both parylene C and differently modified parylene C surfaces. A total of four different functional groups corresponding to different ways of surface modification were considered, namely chloride (−Cl), hydroxyl (−OH), carbonyl (−CHO), and carboxyl ($-COO^-$) groups. The atomistic MD simulations were carried out in an NVT ensemble using GROMACS 4.6 software.

Parallel experiments were performed with chemical vapor deposition (CVD) prepared Parylene C (8 μm of thickness) films, provided by ParaTech Coating Scandinavia AB. To modify the parylene C surface, oxygen plasma treatment was carried out using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany). The oxygen insertion and pore formation have a strong impact on the hydrophilicity of the parylene C film. The changes within the surface were followed by contact angle measurements, using a Surftens universal instrument (OEG GmbH). Static contact angles of water were calculated using Surftens 4.3 Windows image processing software for digital images for the determination of contact angles and surface tension.

The evaluation of cells adhesion and focal contacts were performed with MG-63 osteoblast-like cell line.

Results and Discussion

The MD simulations of water film in contact with variously decorated carbon surfaces were performed to quantify the interactions between water molecules and parylene C surfaces. Initially, the SFE of unmodified parylene C is 43.7 mJ/m^2 and consists mostly of dispersive component (43.1 mJ/m^2) with minimal polar influence (0.6 mJ/m^2) as shown in FIG. 1. Modification of parylene C with oxygen plasma and incorporation of oxygen-containing surface functional groups cause a significant increase of the SFE value to 74.2 mJ/m^2 as well as the dispersive and polar components γ_s^d/γ_s^p ratio. The role of the dispersive component diminishes to 26.5 mJ/m^2, while the polar component becomes dominant with 46.6 mJ/m^2. The experimentally obtained ratio of $\gamma_s^d/\gamma_s^p = 0.5$ is in line with the theoretically determined 60% surface coverage for −OH, while the corresponding ratio of dispersive and electrostatic energies ($E_{dispersive}/E_{electrostatic}$) is 0.56. Additionally, the surface coverage of −OH groups of 60% compares quite well with the previously reported XPS and LDI-MS results [1], for chlorine substitution with oxygen [2]. This agreement reveals that the direct insight obtained from MD *in silico* modelling is predictive not only regarding the optimal surface coverage of functional groups but also regarding the key role of balance between electrostatic and dispersive components. Additionally, the obtained results have the practical consequences in terms of biocompatibility observed as enhanced adhesion and focal contacts formation on oxygen plasma modified parylene C.

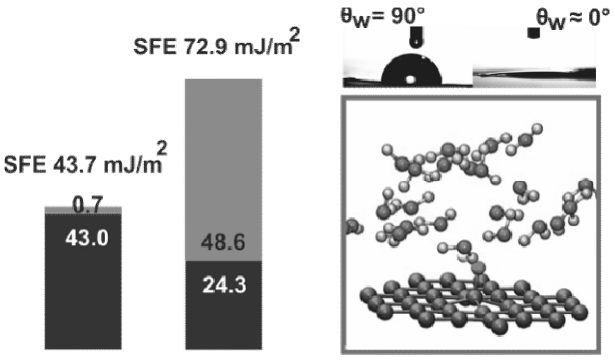

FIG. 1. Summary of the oxygen plasma effect on parylene C SFE and contact angle and typical MD snapshot.

Conclusions

Molecular dynamic simulations give a unique insight into water-material interaction which can be further employed to design polymeric surfaces with intended biological function. Introducing controlled amounts of polar groups of various chemical identities opens new possibilities for more-rational surface modifications and tailoring of implant surface properties for optimal cells−biomaterial interactions.

References

[1] M. Golda *et al., Mater Sci Eng C* 33 (2013) 4221-4227
[2] M. Golda-Cepa *et al.,* ACS Appl Mater Interfaces 9 (2017) 16685−1669361

THE FIRST REPORT ON CHARACTERISATION OF PARTIALLY COVERED SELF-EXPANDABLE METALLIC STENTS IN ESOPHAGEAL CANCER TREATMENT: *IN VIVO* DEGRADATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 22]

Introduction

Squamous cell carcinoma of the esophagus is the fourth cause of death in males and seventeenth in females. There is no change or slight decrease in incidence over the last three decades [1]. More than 50% of patients present with unresectable tumour, progressive weight loss and dysphagia require palliative treatment. Among the many available methods of palliation, stenting, laser therapy, chemoradiation, and photodynamic therapy are considered. However, most often stenting is the method of choice. This is because of technical simplicity, wide availability and immediate alleviation of dysphagia.

Patients requiring stenting are usually diagnosed with III and IV grade dysphagia and significant weight loss. The stents that are currently used, despite relative good tolerance, are not free from side-effects and complications. One of the most common problems associated with stenting is granulation tissue overgrowth and stent obstruction. Coverage with a polyurethane or silicone membrane protects from tumour ingrowth, but overgrowth beyond ends of the stent and granulation tissue formation remains an issue.

The aim of the study was to investigate the impact of long-term usage in the body on physicochemical properties of partially-covered esophageal stents.

Materials and Methods

Structural analysis of 16 partially covered self-expandable metallic stents (SEMS) has been subjected after removal due to their dysfunction. SEMS were implanted because of dysphagia as a result of inoperable esophageal cancer or before chemo-radiotherapy such as bridge to radical surgery treatment. Prostheses have been removed because of their obstruction and recurrence of dysphagia which make oral nutrition impossible for patients.

For the investigations, partially covered SEMS 7–12 cm long and with diameter of 18 mm (Ultraflex Boston Scientific, Natick, MA, USA) were used. For the physicochemical investigation, the obtained stents were cut into 1x1 cm coupons. The morphology of the NiTi stent and polyurethane covering surfaces were evaluated by a Hitachi S-4700 scanning electron microscope (SEM). The properties of polymeric samples were analysed using a TGA/DTA Mettler-Toledo apparatus. The polymeric samples of about 1 mg were placed in an open alumina crucibles. The measurements were carried out in a temperature range of 30–600°C with a heating rate of 5°C min^{-1} at an Ar flow of 50 cm^3 min^{-1} [2]. The changes within the surface of polyurethane were followed by contact angle measurements (CA), using a Surftens universal instrument (OEG GmbH) equipped with Surftens 4.3 Windows image processing software. For each sample, 5 independent 1 µL water drops were applied [3]. ATR-FTIR analyses of the polymeric films were performed on a Spectrum 100 Nicolet 6700 (Thermo Scientific). The spectra were recorded in at least 3 independent spots at the samples in the range 4000–650 cm^{-1}.

Results and Discussion

Structural analysis has been subjected of 16 removed prostheses from patients (3 women and 13 men aged 40-80) which were treated in the course of squamous cell carcinoma (14 patients) and adenocarcinoma of the esophagus (2 patients). Among the treated patients 5 received chemo or chemo-radiotherapy, 5 preoperative chemo-radiotherapy, 6 did not receive treatment.

The SEM observations revealed surface changes on the metal alloy, mostly cracks, on the used esophageal stents when compared to the reference sample. The changes in surface morphology of polyurethanes covers were also visible – the damage of the surface was mostly due to cracks and peeling off 5–10 µm polymeric fragments. The degradation of the polyurethane films was confirmed with ATR-FTIR, indicating the significant loss of intensity at 2930 cm^{-1}, 2860 cm^{-1}, 1740 cm^{-1}, and 1245 cm^{-1} which correspond to the functional groups $–CH_2$, C=O, C-N, respectively. It is worth mentioning that the degradation of polymers was greater at the distal end of the stent. The highly probable reason for that is the more acidic environment nearby the stomach when compared to the proximal end. The bulk changes in the polymer structure before and after stent implantation were compared in terms of melting temperature (T_{melt}). The T_{melt} for the distal end of the investigated stents were shifted of ≈ 20°C towards higher temperatures which indicates significant bulk changes in the polyurethane covers exposed to the human body environment.

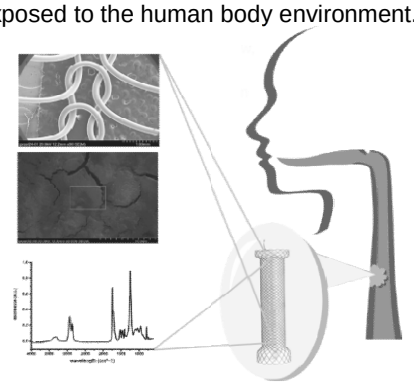

FIG. 1. The overview of the conducted research strategy.

Conclusions

The 16 esophageal stents (Nitinol-polyurethane) were examined after prolonged usage in the body. Physicochemical characterization (SEM, ATR-FTIR, TG/DTA, CA) revealed significant changes in the materials bulk (T_{melt}) and surface morphology as well as surface functional groups (CA, IR). It was concluded, that the degradation strongly depends on the physiological environment (i.e. medical treatment, in-site pH). The main directions for improvement of the stents were pointed out.

Acknowledgments

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References

[1] D.M. Parkin *et al.* Ca Cancer J Clin 55 (2005) 74-108,
[2] M. Golda-Cepa *et al.,* RSC Advances 5 (2015) 48816-48821
[3] M. Golda-Cepa *et al.* Mater Sci Eng C 52 (2015) 273-281

COMPREHENSIVE BIOLOGICAL EVALUATION OF BIOMATERIALS USED IN SPINAL SURGERY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 23]

Introduction
It seems that commonly used biomaterials are sufficiently well characterized allowing the responsible selection of biomaterial for the purpose of spine surgery. However, a thorough analysis of available publications indicates the difficulty of comparing them due to the variety of research techniques and experimental conditions. In addition, there is no information on tissue responses observed at the molecular level, allowing for the identification of mechanisms involved in cell response to exposure to the used biomaterial. For this reason, we have decided to perform a comprehensive biological evaluation of representatives of commonly used biomaterials (Ti6Al4V alloy) and polymer (PEEK Optima).

Materials and Methods
The biomaterials of our interest were materials most commonly used in spine implantation. These are Ti6Al4V ELI alloy and PEEK Optima. Compliance with the relevant ISO/ASTM standards of biomaterials used was confirmed by the certifications provided by manufacturers indicating as implantable implant material. Additionally Ti6Al4V alloy prepared by the selective melting with electron beam technology (Ti6Al4V ELI-EBT) was used in this study [1]. The samples were prepared in the form of discs (8 or 16 mm in diameter) of 3 mm thickness. The surfaces of the samples were finished according to the standards used for the production of implantable medical devices. Metal samples were additionally etched and passivated. The final stages of sample preparation, including washing, double-sleeve packaging and steam sterilization, were conducted in a clean zone. The biological evaluation of the examined biomaterials consisted of the following methods: XTT cytotoxicity test [2], micronucleus test [3], surface colonization by bacteria [4], thrombo-compatibility [5], proteome profile [6], transcriptome profile [7]. As a biological material *E. coli* bacterial cells, EA.hy926 line of endothelial cells and Saos-2 line of osteoblasts were used.

Results and Discussion
The extracts obtained from the studied samples showed no significant cytotoxic and genotoxic characteristics for both types of cells in comparison to control culture.
Both Ti6Al4V ELI and PEEK Optima exhibited high resistance to bacterial colonization while lacking the cytotoxic properties in relation to bacteria. In contrast, Ti6Al4V ELI-EBT was significantly more susceptible to microbial colonization.
Blood platelets adhered to the surface of the studied biomaterials and underwent differentiated activation on these surfaces. On the other hand, flow cytometry analysis showed that the contact with titanium alloys samples results in five times higher spontaneous aggregation of platelets remaining in the whole blood and only two-fold increase in the case of contact with PEEK Optima, although fraction of activated platelets in whole blood, evaluated with specific antibodies, shows only a small increase in comparison to control.
Both transcriptome and proteome profiles show very significant changes in gene and peptide expression in both osteoblasts and endothelial cells resulting from contact with the studied samples. These changes concern numerous cellular metabolic pathways and ongoing analysis of the possible effects of these changes are underway.

Conclusions
Comprehensive biological analysis of biomaterials commonly used in spine surgery shows that the results of standard cytotoxicity and genotoxicity tests, although consistent with the available in literature, do not provide complete information about the suitability of these materials. It should be noted that the porous structure of Ti6Al4V ELI-EBT promotes the microbial colonization of this biomaterial. On the other hand, all investigated biomaterials produce significant changes in the expression of genes and peptides, which can be of great importance in the appropriate selection of materials for personalized medicine.

Acknowledgments
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References
[1] Ciupik L.F., Kierzkowska A., Technology-biomechanical evaluation of metal biomaterials derived by layer technology, Engineering of Biomaterials 93 (2010) 14-18.
[2] Kaminska M, Kuberski S, Maniukiewicz W, Owczarz P, Komorowski P, Modrzejewska Z, Walkowiak B. Thermosensitive chitosan gels containing calcium glycerophosphate for bone cell culture. Journal of Bioactive and Compatible Polymers, 2017; 32(2), 209-222.
[3] Hayashi M. The micronucleus test—most widely used *in vivo* genotoxicity test. Genes Environ. 2016; 38(18), 1-6.
[4] Jakubowski W, Bartosz G, Niedzielski P, Szymański W, Walkowiak B. Nanocrystalline diamond surface is resistant to bacterial colonization. Diamond and Related Materials. 2004; 13(10): 1761-1763.
[5] Walkowiak-Przybylo M, Klimek L, Okroj W, Jakubowski J, Chwilka M, Czajka A, Walkowiak B. Adhesion, activation and aggregation of blood platelets and biofilm formation on the surface of titanium alloys Ti6Al4V and Ti6Al7Nb. Journal of Biomedical Materials Research Part A 2012; vol 100A, 768-775.
[6] Komorowski P, Walkowiak-Przybylo M, Walkowiak B. Early cell response to contact with biomaterial’s surface. J Biomed Mater Res Part B 2016:104B:880–893.
[7] Walkowiak-Przybyło M, Komorowski P, Walkowiak B. Differences in the expression of cell cycle genes in osteoblasts and endothelial cells cultured on the surfaces of Ti6Al4V and Ti6Al7Nb alloys. J. Biomed Mater Res Part A, 2017, 105 (6), 1607-1617.

POLYLACTIDE-BASED COMPOSITES REINFORCED WITH BIODEGRADABLE GLASS FIBERS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 24]

Abstract
This paper presents the method of obtaining biodegradable glass fibers and the results of strength tests of polylactide based composites reinforced with obtained fibers. Polylactide (PLA) is a biodegradable plastic used in both industry and medicine. In medical applications its use is limited due to low mechanical properties. One of the methods to improve the strength parameters is the development of composite materials based on PLA with fibrous fillers. In this work as a matrix, the NatureWorks 4043D polylactide was used. For the reinforcement, the continuous, biodegradable glass fiber was used. These fibers were obtained by direct pulling out of the preform. Their composition is based on the composition of the biodegradable 45S5 glass, but the contents of calcium oxide (CaO) and sodium oxide (Na_2O) have been changed. Also the potassium oxide (K_2O) was added. Composite samples were obtained by flooding the fibers with polylactide dissolved in dichloromethane. Strength tests were carried out using the Zwick/Roell Z010 strength machine. The tensile strength values for composites ranged from slightly less than 11 MPa to almost 20 MPa. For PLA samples it ranged from about 14 MPa to slightly less than 18 MPa. The highest values of tensile strength were obtained by composite samples. Initial studies show that it is possible to improve the tensile strength of PLA samples by incorporating biodegradable glass fibers into their structure.

Keywords: tensile strength, plastic, biodegrability, glass fiber

ANTIOXIDANT ACTIVITY OF NOVEL PCL/BIOACTIVE GLASS COMPOSITES ENRICHED WITH POLYPHENOLIC COMPOUNDS EXTRACTED FROM FRUITS AND LEAVES OF SWEET CHERRY (*PRUNUS AVIUM* L.)

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[ENGINEERING OF BIOMATERIALS 143 (2017) 25]

Introduction

Biomaterials can cause an inflammatory response after implantation in living organism. Initial inflammation is an essential part of the tissue regeneration process since is required to modulate cell recruitment, differentiation and angiogenesis. However, excessive inflammation leads to formation of an adverse environment for regeneration and also to generation of reactive oxygen species (ROS). The ROS act as signalling molecules that upregulate cytokines and other inflammatory mediators that induce further inflammatory cell migration [1,2]. Therefore, biomaterials that can reduce the ROS production in a sustained and controlled manner and thereby modulate inflammatory response may be a useful tool for tissue engineering (TE) applications. An incorporation of anti-inflammatory agents into the biomaterial is a strategy for modulating inflammation [1]. Antioxidants are one of the most promising molecules that show anti-inflammatory effects by neutralizing ROS [3].

The aim of this study is to enrich poly(ε-caprolactone)/bioglass composite films with polyphenols (PPh) extracted from fruits and leaves of sweet cherry (*Prunus avium* L.) in order to combine the bioactive properties of bioglass with the biological activities and health benefits of polyphenols.

Materials and Methods

Conventional solvent extraction of polyphenols from fruits and leaves of sweet cherry was performed in 1,4-dioxane. Bioactive glass particles of the composition of (mol%) $40SiO_2$–$54CaO$–$6P_2O_5$ were synthesized by the use of sol-gel and melt-quenching methods. The polyphenols were introduced into materials directly with solvent-plant extract using solvent-casting method. The static water contact angle was evaluated by sessile drop technique. The amount of PPh present on the surface of materials was determined using Folin–Ciocalteu method. The antioxidant activity of the films was evaluated using DPPH, ABTS, and FRAP tests. Bioactivity of materials was assessed using SEM/EDX and FTIR methods after 7-day immersion in SBF.

Results and Discussion

The results showed that the entrapment efficiency of PPh derived from both fruits and leaves of sweet cherry was 100% for all materials, suggesting no loss of PPh and their activity. The content of PPh on the surface of materials containing leaf and fruit extracts increased in the following order: A2gel/PCL/PPh < A2melt/PCL/PPh < PCL/PPh (FIG. 1a). The reduction in the amount of PPh on the surface of composites can be explained by the adsorption of PPh on the surfaces of glass fillers.

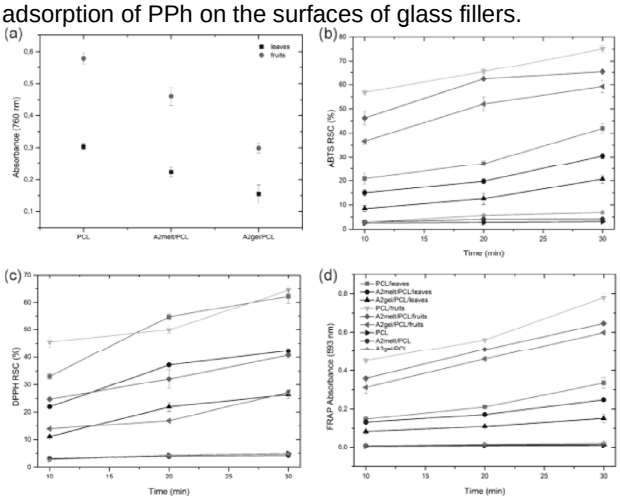

FIG. 1. The amount of PPh on the surface of materials (a), antioxidant activity of PPh-loaded films evaluated using ABTS (b), DPPH (c) and FRAP (d) assays. Results are expressed as mean ± SD (n=3).

After modification with PPh, the films exhibited significantly higher hydrophilicity that can be attributed to the exposition of hydroxyl groups of the PPh present on the surface of films. The antioxidant potential can be clearly ascribed to the presence of PPh in materials (FIGs. 1b-1d). PCL/PPh films showed the highest RSA and reducing potential, while the lowest values were found for A2gel/PCL/PPh materials. The results closely correlated with the content of PPh on the surface of films (FIG. 1a). The surfaces of all composites after soaking in SBF were fully covered with the thick layers rich in calcium and phosphorus. It seemed that layers formed on the films with PPh from fruits contained bigger and well-developed crystal forms. That could have been attributed to the higher concentrations of PPh on the surfaces of these composites. Phenolic hydroxyl groups can promote HCA deposition throughout the interaction with Ca^{2+} ions. The ATR-FTIR spectra of incubated materials were dominated by phosphate and carbonate bands (FIG. 3b) at 558 and 600 cm^{-1} (O–P–O bending mode), 1014 cm^{-1} with a shoulder at 1115 cm^{-1} (P–O stretching mode) and 875 cm^{-1} (CO_3^{2-} bending mode), characteristic for HCA layers.

Conclusions

Materials exhibited excellent *in vitro* bioactivity, improved hydrophilicity and also high antioxidant potential. The use of melt-derived or gel-derived bioactive glass particles and also PPh from leaves and fruits of sweet cherry, caused different PCL-PPh and BG-PPh interactions and therefore affected PPh content on the film surfaces. That, in turn, determined surface wettability, *in vitro* bioactivity, and finally antioxidant activity, providing possibilities for modulating these properties in a wide range. The results suggest that obtained films represent a potential multifunctional biomaterials for bone tissue regeneration.

Acknowledgments

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References

[1] S. Browne et al., Front. Bioeng. Biotechnol. 3(2015) 67
[2] M. Griffith et al., Front. Bioeng. Biotechnol. 4(2016) 71
[3] M.Z. Hosain, et al., Biomater. Sci. Polym. Ed. 27(2016) 290–302

DEPOSITION OF VARIOUS GRADIENT MULTILAYER COATINGS ON Ti-6Al-4V ALLOY USING MW CVD METHODS FOR ORTHOPAEDIC IMPLANTS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 26]

Introduction

Titanium and its alloys are considered to be the most applicable materials for bone implantations [1]. Even though innovative alloys such as *e.g.* Ti-6Al-7Nb have been recently replaced by Ti-6Al-4V alloy, it is still the most widespread alloy for orthopaedic applications. Thus, there is a necessity to modify surface of this material to improve its properties. The functionalized surfaces exhibit better mechanical and biological (e.g. osseointegration and biocompatibility) properties, that are crucial for successful implantation surgery [2,3]. Diamond-like Carbon (DLC) coatings are considered to meet these requirements, but their properties such as modulus of elasticity and residual stress cause unfavourable stress distribution at the substrate-coating interface. Therefore, deposition of gradient layers or doping them with silicon or nitrogen can solve this problem.
In this work, multi-layered nitrogen doped coatings of carbon-hydrogen and/or silicon-hydrogen structure obtained by deposition using Microwave Plasma Assisted Chemical Vapour Deposition method are presented.

Materials and Methods

Samples made from Ti-6Al-4V alloy (3 mm thickness) were mechanically polished, to achieve mirror-like surface, and coated in Plasma Assisted Chemical Vapour Deposition (PA CVD) system, equipped with microwave (2.54 GHz) antenna. Five various processes were performed - TABLE 1.

TABLE 1. Details of experimental series for Ti-6Al-4V modified with application of MW CVD system

Series	Type of coatings	Plasma nitriding
1C	SiCNH/SiCNH-CNH/CNH	No
2C	N^+/SiNH-N^+/N^+	Yes
3C	N^+/SiCNH/SiCNH-CNH/CNH	Yes
4C	N^+/SiNH/SiNH - SiCNH/SiCNH/ SiCNH-CNH/CNH	Yes
5C	SiNH/SiNH - SiCNH/SiCNH/ SiCNH-CNH/CNH	No

Series 2C, 3C and 4C were obtained with application of plasma nitriding process before particular multilayers deposition. While, series 2C was additionally subjected to plasma nitriding after deposition process.
Structure characterization of the resulting coatings in different atomic scale was carried out by SEM and IR spectroscopic method. Hardness and modulus of elasticity were determined by nanoINDENTER® G200 (Agilent technologies, USA) with continuous stiffness measurement. Additionally, preliminary biological study *in vitro* was performed: (*i*) cytotoxicity towards human osteosarcoma MG-63 cell line was evaluated by MTT assay and (*ii*) release of Ti, V, and Al ions to cell culture media was detected by ICP-MS methods. Wettability and surface free energy (SFE) of tested samples were investigated using an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany).

Results and Discussion

As a result of application of MW CVD method smooth modified surfaces of Ti-6Al-4V alloy with characteristic granular structure have been obtained - FIG. 1.

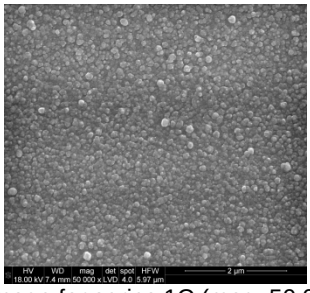

FIG. 1. SEM image for series 1C (mag. 50 000 x).

After plasmochemical functionalization the tested series (1C - 5C) showed surface roughness R_a in the range of 4.82 ÷ 3.35 nm, with the highest value observed for modification 1C. IR spectra confirmed the presence of typical atomic groups for the resulting structures. It was showed that application of gradient coatings is a diffusion barrier for metal ions released from the modified alloy surface. All types of plasma modifications significantly changed of Young modulus of the functionalized substrates (up to *ca.* 1.2 µm from surface) in comparison with the untreated Ti-6Al-4V alloy. For instance, low hardness value of tested samples after modification is connected with the least dense, granular structure.

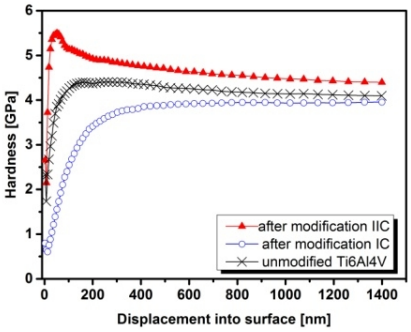

FIG. 2. Hardness profile of the unmodified and selected modified Ti6Al4V alloy in plasma conditions

Conclusions

In this investigation, it was demonstrated that for the majority of plasma-based surface modifications physicochemical properties such as roughness, wettability, and hardness were improved. The most significant improvement was observed in case of series 1C, consisted of deposition on the top CNH layer without pre- and post- plasma nitriding process. This, in turn, results in low values of hardness and Young modulus of the modified surfaces.

Acknowledgments

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References

[1] T. Kokubo et *al*., Acta Biomater. 44 (2016) 16-30.
[2] K. Kyziol et *al.*, Wiley-Scrivener, (2017) 457-490.
[3] X. Liu et *al.*, Mater. Sci. Eng. R Reports. 70 (2010) 275–302.

ACRYLIC COMPOSITE MATERIALS MODIFIED WITH BEE POLLEN FOR BIOMEDICAL APPLICATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 27]

Introduction

In recent years hydrogels, due to their biocompatibility, cell-controlled degradability, and intrinsic cellular interaction have become one of the most important class of materials used in medicine. Nowadays, hydrogels play an important role in dynamically developing field of tissue engineering where they are used as a three-dimensional porous scaffolds to guide the growth of new tissues, as an implantable devices, in cartilage wound healing, drug delivery systems, wound dress and bone regeneration. The structure and functions of those polymeric materials can be prepared in a precisely controlled way. To foster new tissue formation hydrogels must meet a number of criteria that include physical (e.g. degradation and mechanics) and biological (e.g. cell adhesion) guidelines [7]. Hydrogels are made from natural polymers (e.g. collagen, chitosan, keratin) as well as synthetic polymers (e.g. poly(vinyl alcohol), PVA, poly(ethylene glycol), PEG, poly(acrylic acid), PAA [1].

Poly(acrylic) acid (PAA) has been intensively examined by many scientist in the context of biomedical applications, due to its biocompatibility, pH-sensitivity, solubility in water and aqueous solutions of inorganic salts and simultaneous lack of solubility in most organic liquids. In neutral and alkaline solution acidic carboxyl groups of PAA are ionized and this leads to electrostatic repulsion of the polymer chains. Over recent years, various PAA-based materials for the use in tissue engineering have been proposed [2].

Nowadays, polymeric hydrogels are mainly combined with different type of nancompounds (e.g. graphene oxide, carbon nanotubes, gold–silica nanoshells, nano-hydroxyapatite, ultra-high-molecular-weight polyethylene nanofibres), biologically active proteins or peptides, and reinforced with inherently biocompatible particles (e.g. hydroxyapatite, HAp). HAp is the inorganic component of bones and teeth forming their hardness and strength. HAp due to its porosity, bioactivity, biocompatibility and ability to form a good connection with the living tissues is willingly used as a component of different types of biomaterials, especially in dental and orthopaedic surgery as a filled material for biocompatibility matrix [3].

In this research paper, we describe in detail the preparation, characterization and biocompatibility studies of three-dimensional hydrogel composite material prepared from acrylic acid, particles of HAp and agar (as a stabilizing agent), cross-linked by poly(ethylene glycol) diacrylate (PEGDA) under microwave irradiation and modified by different amounts of bee pollen.

Materials and Methods

The aim of this paper is to present the influence of bee pollen on the physicochemical and in vitro properties of poly(acrylic acid) hydrogel composites enriched with hydroxyapatite and modified with bee pollen as a prospective materials for biomedical application with beneficial features including good osseointegration and anti-inflammatory effect.

Results and Discussion

Phase and chemical composition of hydroxyapatite synthesized by wet-precipitation method was confirmed by means of XRD and FT-IR techniques. Proposed materials were investigated towards in vitro properties by immersion in incubation fluids including artificial saliva, Ringer's solution and distilled water and composites swelling ability was determined. Additionally, the chemical structure of the polymer matrix composites was confirmed by infrared spectroscopy with Fourier transformation. Moreover, to characterize composite degradation process in 21 days incubation FT-IR technique was employed. Hydrogel composite before incubation (a) and after 21 days immersion (b) are shown in FIG. 1. In order to describe bee pollen feature both scanning electron microscopy and X-ray fluorescence spectrometry were used. Presented research revealed that hydroxyapatite, as well as poly(acrylic acid) undergo biodegradation during in vitro test. Moreover, matrices degradation results in incubation fluids pH decrease associated with anionic nature of poly(acrylic acid), which is further enhanced by bee pollen release. The strongest pH drop effect was observed for Ringer's solution. Increase in conductivity of distilled water confirmed composites degradation process.

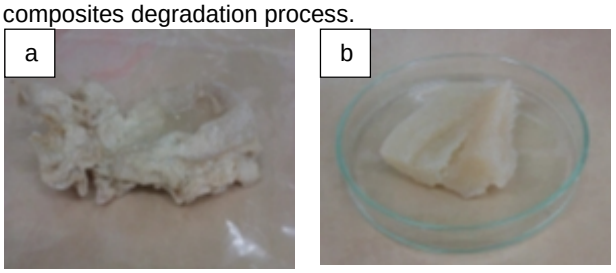

FIG. 1. Hydrogel composite before incubation (a) and after 21 days immersion (b).

Conclusions

Prepared composite materials present good sorption capacity against different fluids used in biomedical testing, such as Ringer's solution and artificial saliva. In vitro tests performed in this research proved that investigated materials are stable in body-simulated conditions during 21 days incubation. Given the fact that acrylic hydrogel materials are already applied as a wound dressing devices, further investigations should be focused on adoption this structure in the field of biomaterials supporting bone regeneration, which can be achieved by bioactive phosphates incorporation. It can be presumed that so-modified hydrogels would possess such beneficial properties and would support osseointegration.

Acknowledgments

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References

[1] Parlato M, Reichert S, Barney N, Murphy WL, Macromol. Biosci.14: (2014) 687
[2] Nho YC, Park JS, Lim YM, Polymers 6 (2014):890
[3] Li Z, Su Y, Xie B, Wang H, Wen T, He C, Shen H, Wu D, Wang D ,J. Mater. Chem. B (2013) 1:1755

MAGNESIUM BIODEGRADABLE IMPLANTS COATED WITH POLYLACTIC ACID

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[ENGINEERING OF BIOMATERIALS 143 (2017) 28]

Introduction

Magnesium alloys and polylactic acid polymer (PLA) coatings are optimum materials for biodegradable implants [1,2], but their combination is limited because of the low adhesion of PLA on Mg alloys. Plasma electrolytic oxidation (PEO) is a valuable surface modification technique for Mg alloys to promote adhesion of coatings, but limited studies have systematically evaluated this effect.

In the present report, dipping method has been used to deposit a PLA coating on AZ31 Mg alloy to tailor its corrosion properties. Furthermore, the surface of the alloy has been modified by PEO [3] to produce a porous coating on AZ31 Mg alloy as an intermediate layer to improve the adhesion strength between PLA layer and the magnesium alloy, and at the same time enhancing the properties of the PLA coating.

The influence of dipping parameters (number of layers, withdrawal speed and polymer concentration) in several properties of the coating (mass increment, thickness, roughness, adhesion and resistance polarization) has been evaluated by using the Taguchi design of experiment (DOE) method [4]. In addition, the effect of the PEO treatment has been evaluated for the different coating parameters. Finally, the corrosion behavior of the different systems was also studied.

Materials and Methods

AZ31 commercially available magnesium alloy was used as the base substrate and PLA was used as the coating material. PLA was dissolved in chloroform and coated on the samples using the dip-coating technique. PEO treatments were carried out using alternating current (AC) voltage. The large number of variables involved in dip coating processes, are reduced to a limited number of experiments by the Taguchi design of experiment (DOE) method. This methodology was used to analyze the influence of the dip coating conditions (number of layers, withdrawal speed and polymer concentration) in the main characteristics of the coatings (mass increment, thickness, rugosity, adhesion and resistance polarization). Every parameter was evaluated for three levels (different values), as we can see in TABLE 1:

TABLE 1. Manufacturing factors and values used for each

FACTORS	LEVEL 1	LEVEL 2	LEVEL 3
LAYERS	3	4	5
SPEED (cm/min)	8	35	75
CONCENTRATION (%w/w)	1.23	2.5	6

In terms of the coating, concentration of PLA in the dipping solution seems to be the most important parameter controlling the properties of the coatings.

The results show that PEO treatment did not increase the adhesion strength of the PLA coating, although the corrosion potential and polarization resistance were increased.

Results and Discussion

The potentiodynamic polarization curves of the samples are shown in FIG. 1 and the corresponding electrochemical data are listed in TABLE 2.

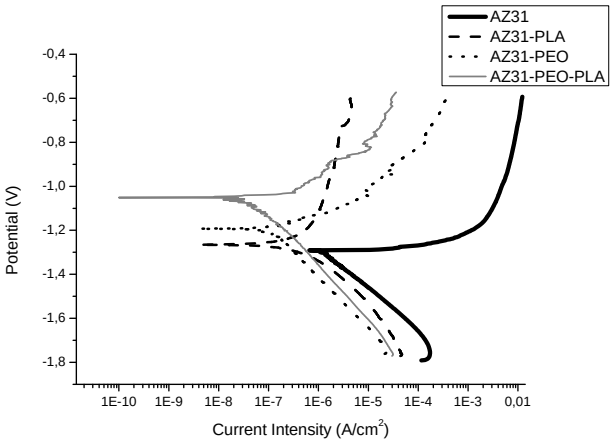

FIG. 1. Potentiodynamic polarization curves of the samples exposed to Hanks solution.

The corrosion potential value of double coated sample is higher than for single coated and uncoated Magnesium sample (FIG. 1). There is also a reduction in the corrosion current of the double-coated sample compared to uncoated Mg alloy (TABLE 2).

TABLE 2. Electrochemical data for the coated and uncoated samples.

	Potential (V)	Current Density
AZ31	-1.3232	1.48E-06
AZ31-PLA	-1.2643	2.38E-07
AZ31-PEO	-1.1897	9.27E-08
AZ31-PEO-PLA	-1.0928	5.96E-08

Conclusions

PEO treatment did not increase the adhesion strength of the PLA coating, although the corrosion potential and polarization resistance were increased. In terms of the coating, concentration of PLA in the dipping solution seems to be the most important parameter controlling the properties of the coatings.

Acknowledgments

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References

[1] A. Alabbasi, M.B. Kannan, C. Blawert, Materials Letters 124 (2014) 188–191
[2] H.R. Bakhsheshi-Rad, E. Hamzah et al., Vacuum 125 (2016) 185-188
[3] M. Mohedano, B.J.C. Luthringer et al., Surface & Coatings Technology 315 (2017) 454-467
[4] B. Torres, C. Taltavull, A.J. López, et al. Surface & Coatings Technology 261 (2015) 130-140

INORGANIC NANOPARTICLES ON BACTERIA: THE KEY FACTORS RULING THE INTERACTION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 29]

Introduction

Nanoparticles (NPs) of various materials (metals and metal oxides), shapes (rods, plates, cubes, etc.) and sizes (1-100 nm) exhibit different physical and chemical properties, including adsorption of biomolecules and surface functionalization. It allows to adjust specific NPs for better NPs-bacteria interactions. The interactions between bacteria cell wall and NPs are influenced by several factors, e.g. size, charge and pH, surface functional groups, adsorbed peptides/proteins and other potential adsorbing compounds which all have an effect on the aggregation degree and the dispersion of NPs. In the literature there are usually pointed out two key factors responsible for the NP-bacteria cell wall adhesion: chemical affinity and NPs surface area [1]. There are some studies revealing size-specific affinity as NPs attached to the bacterial membrane within a narrow size distribution, e.g. for the interaction of *E. coli* with Ag NPs the optimal size is 5.4 ± 0.7 nm [2]. NPs-bacteria interaction can be obtained by surface adsorption and/or internalization, controlled by the surface charge and cell wall structure [3], because bacteria cell wall has a complex chemistry and significantly differ between Gram-positive and Gram-negative bacteria strains. Usually Gram-positive bacteria are highly negatively charged due to the exposure of teichoic acid brushes on the cell wall surface. The aim of the work was to investigate the key factors ruling the adhesion of various inorganic NPs on typical bacteria strains.

Materials and Methods

The nanoparticles of cryptomelane (KMn_8O_{16}), cobalt spinel (Co_3O_4), ceria (CeO_2) and α–hematite (Fe_2O_3) were synthesised with standard hydrothermal and/or precipitation methods. The structures of synthesised materials were characterized by Raman spectroscopy and X-ray diffraction. The shapes of NPs were determined with transmission electron microscopy and NPs size distribution with Nanoparticle Tracking Analysis (NTA). Bacterial cell surface (*S. aureus, S. maltophilia*) electric net charges were characterized by the zeta potential, which is the electrical potential of the interface between the aqueous solution and the stationary layer of fluid attached to the bacterial cell. The isoelectric point of NPs and bacteria in water suspensions were calculated based on zeta potential measurements.

Results and Discussion

It was revealed, that using different synthesis methods it is possible to obtain inorganic nanoparticles with different size and shape. The preliminary results showed that the interaction between nanoparticles of typical functional oxides (CeO_2 and Fe_2O_3) and bacteria cell walls (*S. aureus, S. maltophilia*) depends on several factors, especially the concentration of NPs and bacteria suspension, time of incubation and electric charges of microorganisms and NPs. The measured zeta potential values of ceria and investigated bacteria strains are presented in TABLE 1. The bacteria–NPs surface interactions can be interpreted in terms of mutual interactions – electrostatic (Coulomb) and dispersive (van der Waals) which provides the basis for the energetic profile in DLVO (Derjaguin–Landau–Verwey–Overbeek) theory [4]. This approach can be used for controlled deposition of nanoparticles on bacteria. The proposed model of bacteria interaction with nanoparticles surface is presented in FIG. 1 showing the main three steps in the interaction upon NPs approaching the bacteria cell surface.

TABLE 1. Energetic model of nanoparticles-bacteria interactions.

NPs/bacteria strain	ζ potential (H_2O) / mV
CeO_2	+28
S. aureus	-13
S. maltophilia	-4

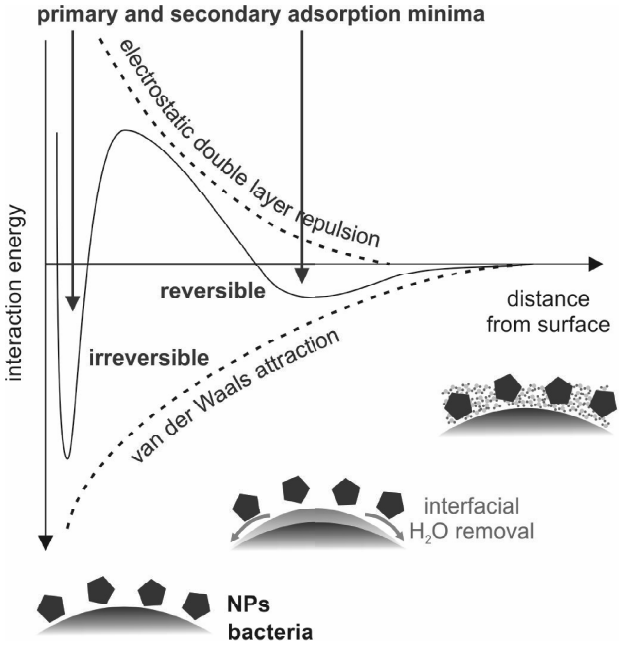

FIG. 1. Energetic model of nanoparticles-bacteria interactions.

Conclusions

It was found out that the electrostatic interactions play a key role in NPs adsorption on the bacteria surface. Specifically, positively charged nanoparticles are easily adsorbed on negatively charged bacteria cell walls. Such findings may play an important role in several applications, e.g. designing antimicrobial surfaces and biosynthesis of nanoparticles. The studies provide basis for the in–depth investigations of NPs-bacteria interactions on both levels, molecular and macroscopic.

Acknowledgments

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References

[1] S. Agnihotri, M. Soumyo *et al.*, RSC Advances 4.8 (2014) 3974-3983.
[2] J. Crespo, J. García-Barrasa *et al.*, Journal of Nanoparticle Research 14 (2012), 1-13.
[3] L. Hall-Stoodley, J. W. Costerton *et al.*, Nature reviews microbiology 2.2 (2004) 95-108.
[4] M. Golda-Cepa, K. Syrek *et al.,* Materials Science and Engineering: C 66 (2016) 100-1205.

BACTERICIDAL TiO_2 LAYERS DOPED WITH Cu, Zn, AND ZnO

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[ENGINEERING OF BIOMATERIALS 143 (2017) 30]

Introduction

The constant presence of chemical antimicrobial substances in our environment poses the risk of selecting strains of bacteria that are resistant to the bactericidal agents. At the same time, many research groups point to the need to increase the protection of the antibacterial surface on commonly used materials. Particularly important surfaces appear to be exposed to large numbers of people at places such as public transport or hospital facilities, which may also lead to the spread of pathogenic microorganisms.
One of directions of research is to develop coatings with antibacterial properties based on the action of inorganic compounds such as metal ions or metal oxides. This reduces the use of antibiotics or bacteriostats, reducing the risk of increased resistance to the pharmaceuticals used [1,2].
In this work, coatings produced by anodic oxidation using electrolytes containing ionic germicidal compounds were used.

Materials and Methods

The samples of Ti6Al4V were prepared in the form of discs of 16 mm in diameter and of 3 mm thickness. The surfaces of the samples were prepared according to the standards used for the production of implantable medical devices.
Anodic acid solutions (H_3PO_4, H_2SO_4, HF) and antimicrobial compounds ($CuCl_2$, $ZnSO_4$, ZnO) were used as the working electrolytes for the anode oxidation process.
Doped TiO_2 layers were characterized by measurement of: contact angle and roughness, as well as by analysis with SEM microscopy and EDS spectrometry. Verification of bactericidal potential was carried out using fluorescence microscopy and flow cytometry, as well as the susceptibility test for bacterial colonization of doped TiO_2 layers [3].

Results and Discussion

The efficiency and effectiveness of TiO_2 layers deposition on the titanium surface were assessed by the use of variable operating conditions, i.e. - application time, working electrolyte composition, and current. This allowed us to optimize the proper conditions for obtaining expected continuity and layer thickness on the surface of titanium samples. At the same time, effects of these parameters on bactericidal properties were analyzed - we showed that there are differences in the type of acid used in the electrolyte.
An essential part of this work was testing of antibacterial effect substances introduced into the layer during the anode oxidation process, it means Cu^{2+}, Zn^{2+}, and ZnO in the form of nanopowder. The range of used concentrations was from 0.1 to 1mM in the working electrolyte, and the results show a correlation of antimicrobial protection with the level of added dopants. EDS analysis confirmed the presence of doped substances in the produced layers.

Conclusions

Currently, there is a trend in reducing the level of antibiotics used as protection against the development of undesired bacterial biofilms [4]. The research demonstrates the potential in this respect of technology based on the addition of ionic additives that increase the level of protection against surface colonization and the development of microorganisms.

References

[1] Bazaka K, Jacob MV, Chrzanowski W, Ostrikov K Anti-bacterial surfaces: natural agents, mechanisms of action, and plasma surface modification. RSCAdv., 2015, 5, 48739–48759
[2] Alfa MJ, Lo E, Olson N, MacRae M, Buelow-Smith L. Use of a daily disinfectant cleaner instead of a daily cleaner reduced hospital-acquired infection rates. Am. J. Infect. Control 2015, 43 (2), 141–146
[3] Jakubowski W, Bartosz G, Niedzielski P, Szymański W, Walkowiak B. Nanocrystalline diamond surface is resistant to bacterial colonization. Diamond and Related Materials. 2004; 13(10): 1761-1763.
[4] Świątek L, Olejnik A, Grabarczyk J, Jedrzejczak A, Sobczyk-Guzenda A, Kaminska M, Jakubowski W, Szymanski W, Bociaga ,D Multi-doped diamond like-carbon coatings (DLC-Si/Ag) for biomedical applications fabricated using the modified chemical vapour deposition method. Diamond & Related Materials 2016 67, 54–62

POTENTIAL DEGRADATION OF BONE CEMENT USED IN MAXILLO-FACIAL SURGERY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 31]

Introduction

Bone cement is a biomaterial, which is used as a material for filling bone defects, stabilizing complicated fractures and fixing implants. As a result of various types of bone damage or tumors, it is used in maxillo-facial surgery. In this environment, bone cement is exposed to stress, aggressive solution and corrosive agents in the case of bonding metal implants [1-5].
The purpose of this study is to examine its potential degradation in oral cavity. The aim of selected research is to observe the quality of obtained bone cement and checking the effect of the artificial saliva solution on it.

Materials and Methods

The research was carried out on bone cement. It was prepared by manual mixing at 2 spins per seconds and formed into square-shaped samples of 20x20 mm and a thickness of approximately 1.5 mm (FIG. 1). In addition, parts of the samples were applied to 2 mm thick titanium plates.

FIG. 1. Bone cement sample.

The following research were carried out on: topography of the surface using scanning electron microscope, wetting angle of the surface using optical tensiometer, absorption of the solution for 1h and 24h, corrosion in the artificial saliva solution.

Results and Discussion

Surface topography was observed using scanning electron microscope. The average number of pore on the surface of 700x1200 μm was 18x and their approximate size was 10-30 μm. An example of topography (magnification 100x) and pore (magnification 2000x) is shown in FIG. 2.

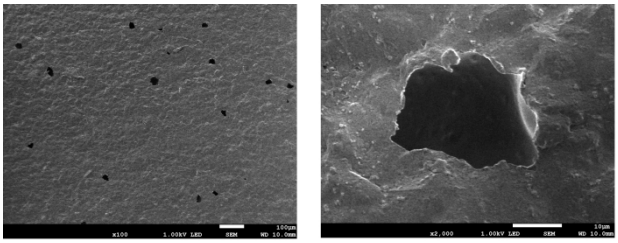

FIG. 2. Topography of bone cement and sample pore.

The average contact angle 61.3° was determined at the moment of falling a drop and next decreased to 58.2° after 5 seconds, and after 10 seconds to 56.7°. A sample test result is shown in FIG. 3.

FIG. 3. Sample contact angle test.

Absorption of the artificial saliva solution by immersion in bone cement samples and retention at 37° were investigated. After 1h the average weight of the samples increased by 0.0034 grams, and after 24 h by 0.0067 grams. The samples were dried for 1 h at 50°C and the hydration degree (Ha) was calculated, by using following Eqs. [6]:

$$H_a\,(\%) = \left(\frac{m_w - m_f}{m_o}\right) x100$$

Calculated the average hydration degree was 0.68% after 24 hours immersion in solution and drying.

A corrosion test was performed in the artificial saliva solution and the average corrosion current was determined to 6.72 A/cm^2 and the average corrosion potential was -429.57 V. Comparatively, for titanium samples without bone cements coverage, the average corrosion current was 26,246,72 A/cm^2 and the average corrosion potential was -386.5 V. Surface topography (magnification 100x) and pores (magnification 2000x) were observed using the SEM microscope – FIG. 4.

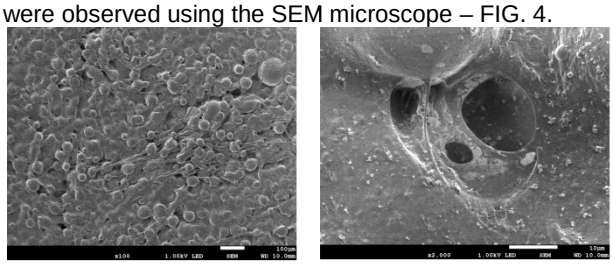

FIG. 4. Topography of bone cement after corrosion test.

Conclusions

Porous bone cement with a relatively distribution of pores was obtained,
The wetting of the resulting bone cement shows, that it is hydrophilic and will provide adequate adhesion of water, protein and osteoblast, which will allow the osteointegration process,
The water absorption by the bone cement is relatively small after 24 h – Ha=0.68%, which means that there is no loss of mechanical properties due to the presence in the aquatic environment,
The coverage of metal implants with bone cement weakens its corrosion in aggressive environment, but unfortunately the effect of corrosive agents is to "dissolve" the top layer of bone cement.
It is assumed, that bone cement can be used in maxillo-facial surgery.

References

[1] F. Michael, M. Khalid et al., Mater. Sci. Eng C, 67 (2016) 792-806.
[2] I. Koh, A. Lopez et al., J. Mech. Beha. Biomed. Mater., 51 (2015) 50-60.
[3] S. Kim, S. Jeon et al., J. Ind. Eng. Chem., 18 (2012) 128-136.
[4] H. Tan, S. Guo et al., Acta Biomater., 8 (2012) 2166-2174.
[5] M. Miola, A. Bistolfi et al., Mater. Sci. Eng. C, 33 (2013) 3025-3032.
[6] J.Slane, J. Vivanco et al., J. Mech. Beha. Biomed. Mater., 29 (2014) 451-461.

DECORATION OF ELECTROSPUN FIBERS WITH CHITOSAN NANOPARTICLES LOADED WITH ESSENTIAL OILS FOR BACTERICIDAL AND ANTI-INFLAMMATORY APPLICATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 32]

Introduction

Infections are the most common postsurgical complications, which present serious threat to patients life [1]. In addition, more and more bacteria strains are becoming resistant to antibiotics [2]. Therefore, attention of researchers has been recently attracted by essential oils (EO) – a wide group of substances, from which some oils exhibit very good antibacterial and anti-inflammatory properties. Especially carvacrol (CRV) and thymol (TM) possess high activity against *E.coli, S. aureus* and *P. aureginosa* [3] – strains, which inhabit our skin and are mostly responsible for causing infections [1]. What is more, traditional bandages accumulate blood and other liquids from wound, which are potential source of nutrients for bacteria. This may result in development of infection and cause serious problems for human health.
In this study, we focused on designing of an active wound dressing made of eletrospun polymeric fibers, decorated with chitosan nanoparticles loaded with a bioactive agent (essential oil: CRV or TM).

Materials and Methods

Preparation of oil-loaded chitosan particles
All chemicals were purchased from Sigma Aldrich and used as received. CRV or TM (150 mg, pure grade) was mixed with Tween 80 (106 µl, HLB 15), then 15 ml of 0.5% (w/v) low average molecular weight chitosan (50-190 kDa) in 1% (v/v) acetic acid was added. Mixture was vortexed for ~30 s and 5 ml of 0.3% (v/v) tripolyphosphate (TPP) solution was added dropwise and stirring was carried out for 30 min in 25°C with stirring speed 700 rpm. Particles were collected by centrifugation at 13 000 rpm (10 min) in ambient temperature, washed with 20 ml of 0.5% Tween80 solution and twice with distilled water. Supernatants were collected to determine encapsulation efficiency. Obtained particles were dispersed in 1 ml of distilled water and kept in 4°C.

Characterisation of loaded chitosan particles
Dynamic light scattering was used to measure hydrodynamic diameter and Zeta potential of obtained spheres. SEM imaging was carried out to determine morphology and size of particles. UV-vis absorption spectra of SN were measured (abs. max.: 247 nm CRV and TM). Concentrations of EO in supernatants were calculated from calibration curve. IR spectrophotometry was carried out to confirm successful loading of EO into polymer matrix. Also, thermogravimetry (TGA) was performed in temperature range of 25-600°C with heating rate of 10°C/min and with N_2 flow rate of 50 ml/min.

In vitro release of oils from chitosan nanoparticles
Essential oils were released using dialysis membranes in PBS with 0.1% (v/v) Tween80, pH = 7.4 in 37°C. After each period of time, samples in dialysis bag were moved into new container with fresh PBS. Samples of PBS were measured using UV-vis.

Electrospinning and decoration of fibers with NPs
Polycaprolactone (PCL) was dissolved in mixture of dichloromethane and dimethylformamide obtaining 10% solution. Solution of polymer and suspension of chitosan nanoparticles loaded with EO in ethanol were electrospun simultaneously on rotating collector (100 rpm), with pumping rates of 1 ml/h (PCL) and 2 ml/h (NPs). Nozzles were swiping above the collector (3 cm/s) to prevent separation of polymer and particles.

Results and Discussion

Obtained particles exhibited hydrodynamic diameter of 290 nm (DLS) and incipient stability with zeta potential around 20 mV. SEM imaging has shown that dry NPs possessed spherical shape, regular size distribution and diameter *ca.* 90 nm (FIG. 1, left). Mean encapsulation efficiency of EO was 25% and 28% for CRV and TM respectively. *In vitro* release studies showed release efficiency around 31% and 22% respectively for CRV and TM. Encapsulated EO were released in initial burst effect lasting 6-8 hours (FIG. 2, right). SEM imaging confirmed successful decoration of electrospun PCL fibers with chitosan nanoparticles loaded with EO (FIG. 1, right).

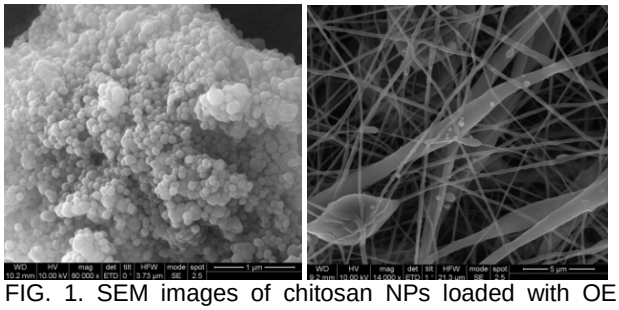

FIG. 1. SEM images of chitosan NPs loaded with OE (left) and decorated with them electrospun PCL fibers (right).

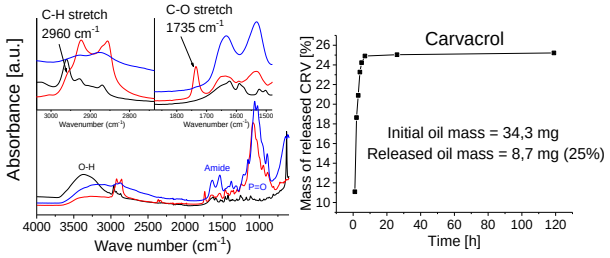

FIG. 2. IR spectra (left) and exemplary release profile (right).

Conclusions

Chitosan nanoparticles loaded with EO were obtained by ionic gelation technique. Loading of EO into chitosan NPs was confirmed by UV-vis, IR (FIG. 1, left) and TGA. Encapsulation efficiency was *ca.* 25%. Release rate can be considered as effective, as all of released drug would be released during time of using single wound dressing.
Simultaneous electrospinning of PCL fibers and chitosan particles loaded with EO is an effective method to functionalise fibers with polymeric NPs.

References

[1] M. Montewka *et al.,* Post. Microbiol., (2012) 227-235.
[2] M. Bonyadian et al., Vet. Res. Forum., 5 (2014) 29-34.
[3] A. Guarda et al., Int J Food Microbiol., 146 (2011) 144-150.

RADIATION–INDUCED SYNTHESIS OF POLYMERIC NANOGELS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 33]

Introduction

The continuous need for new polymeric entities for biomedical application calls for a careful investigation of some dimensional aspects and topological features of polymers. In this context, several methodologies have been reported for the synthesis of nanogels. According to Encyclopedia of Nanotechnology [1] definition of these polymeric nanoparticles may be directly derived from definition of polymeric gel, i.e., a two-component system consisting of a permanent three-dimensional network of linked polymer chains, and molecules of a solvent filing the pores of this network. Based on this approach polymeric nanogels are classified as particles of polymer gels with colloidal properties, having the dimensions in the order of nanometers. One can also distinguish bigger analogues of micrometer size called respectively microgels. Another way of defining polymeric nano- and microgels is based on the specific form of these particles: all the chain segments are linked together, thus being a part of one macromolecule. That is why nano- and microgels as internally cross-linked polymeric chains are considered as a distinct type of macromolecules [2].

Results and Discussion

A number of synthetic routes have been developed for synthesis of nano- or microgels of different chemical structure, architecture and properties. These methods can be divided into two groups. The first one encompasses the techniques based on simultaneous polymerization and cross-linking (sometimes called cross-linking polymerization), where the substrates are monomers or their mixtures. The second group includes methods based on cross-linking of macromolecules where the starting material is not a monomer but a polymer. Works of Burchard and co-workers [3] give examples of microgel synthesis based on intramolecular cross-linking of polymeric chains. It has been shown that reaction of intramolecular cross-linking can be carried out with water-soluble polymers in dilute solutions (polymer concentration must be low enough to avoid intermolecular cross-linking) using a cross-linker capable of reacting with the chains functional groups (e.g.: –OH, –COOH or other). The weight and dimensions of the final product depend on the linear polymer chain weight and the cross-linker concentration which influences the internal cross-link density.

An alternative method of intramolecular cross-linking initiation is the use of ionizing radiation as proposed by Rosiak and coworkers [4,5]. Its undoubted advantage is the fact of avoiding any additives as the reaction can be performed in a pure polymer-solvent system. This method is particularly well suited for the preparation of products for biomedical applications.

When a polymer is subjected to ionizing radiation (typically gamma rays or high-energy electrons) in a dilute aqueous solution, most of the radiation energy is absorbed by water. As a result, short-lived reactive species, namely OH radicals, H-atoms and hydrated electrons, are formed. The initial radiation chemical yields of these species in Ar-saturated solutions are $G(OH) = 2.8 \times 10^{-7}$ mol J^{-1}, $G(H) = 0.6 \times 10^{-7}$mol J^{-1}, and $G(e_{aq}^-) = 2.7 \times 10^{-7}$ mol J^{-1}. Since electrons are inert towards simple, aliphatic water-soluble polymers, they can be converted into additional OH radicals by saturating the solution with N_2O. Hydroxyl radicals and hydrogen atoms are capable of abstracting hydrogen atoms from macromolecules, generating polymer radicals.

Macroradicals generated during irradiation of dilute aqueous polymer solution react in a number of different one-radical (chain scission, hydrogen transfer) and two-radical (cross-linking and disproportionation) reactions. Recombination may occur either between two radicals localized on separate macromolecules or between two radicals within the same chain. The proportion between recombination and disproportionation reactions is set by the radical structure and the possibility to control this parameter is usually very limited.

The basic reaction for the formation of nanogels upon irradiation of a polymer in solution is intramolecular cross-linking of polymer radicals. Since there is always a competition between this process and other reactions, the basic aim in the design of a synthetic procedure is to choose such conditions that promote intramolecular cross-linking and reduce the yield of the unwanted side processes, especially degradation and intermolecular cross-linking.

It has been shown that one of the most important factors in such case is the average number of radicals present simultaneously on each polymer chain. High numbers of radicals can be generated as a result of short but intense pulses of fast electrons. Detailed discussions on this topic have been given by Rosiak and his group [4-7]. If the average number of radicals per chain is lower than one, the single radical that does not find a reaction partner within its own macromolecule, can undergo scission or rearrangements that may change its nature and life-time or can recombine with a radical on a neighboring polymer chain. As a result of that, the macromolecules become linked together, the average molecular weight increases and, finally, a macroscopic, ‘‘wall-to-wall’’ hydrogel is formed. On the other hand, when the number of radicals per chain significantly exceeds one, intramolecular cross-linking dominates, leading to the formation of nanogels. Such reaction conditions can be achieved by lowering the polymer concentration and increasing the dose per electron pulse.

In this work summary on radiation induced intramolecular crosslinking will be given including information on parameters influencing synthesis of nanogels, mechanism and kinetics of this process, physico-chemical properties of irradiation products as well as preliminary application attempts.

References

[1] Ulanski, P., Rosiak, J.M., 2004. Polymeric Nano/Microgels, in: Encyclopedia of Nanoscience and Nanotechnology

[2] IUPAC, 1997. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book").

[3] Brasch, U., Burchard, W., 1996. Macromolecular Chemistry and Physics 197, 223-235

[4] Ulanski, P., Janik, I., Rosiak, J.M., 1998. Radiation Physics and Chemistry 52, 289-294.

[5] Ulanski, P., Rosiak, J.M., 1999.Nucl. Instrum. Nuclear Instruments and Methods in Physics Research B 15, 356-360.

[6] Kadlubowski, S., Grobelny, J., Olejniczak, W., Cichomski, M., Ulanski, P., 2003 Macromolecules 36, 2484-2492.

[7] Kadlubowski, S., Ulanski, P., Rosiak, J.M., 2012 Polymer 53, 1985-1991.

CALCIUM PHOSPHATE NANOPARTICLES FOR GENE TRANSFER AND SILENCING - A 2D AND 3D CELL CULTURE MODEL STUDY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 34]

Introduction
Gene transfer as a tool to control internal processes in living cells is of great importance in cell biology and biomedicine. Calcium phosphate (CaP) nanoparticles may serve as nucleic acid carriers with high biocompatibility as CaP is a natural component of the human body in bone and teeth [1]. Cell culture models are essential tools to investigate many intracellular processes, including gene transfer and silencing, as well as particle uptake [2]. When compared to a traditional monolayer 2D cell culture model, a spatial 3D cell culture model, due to its higher complexity and more realistic parameters, better reflects the natural cytoarchitecture of tissues, serving as a bridge between *in vitro* and *in vivo* studies [3].

Materials and Methods
CaP nanoparticles were synthesized, stabilized with poly(ethyleneimine), loaded with nucleic acids (DNA/siRNA) and coated with a silica shell. Afterwards they were purified by ultracentrifugation and characterized by dynamic light scattering, nanoparticle tracking analysis and scanning electron microscopy [4]. HeLa cells were transfected with DNA-loaded CaP nanoparticles. For gene silencing in HeLa-EGFP cells, siRNA-loaded CaP nanoparticles were applied. In both cases, the gene transfer efficiency was determined by transmission light microscopy and fluorescence microscopy [2]. The uptake of CaP nanoparticles by HeLa cells was studied by confocal laser scanning microscopy. The cytotoxicity of the nanoparticles was evaluated with a live/dead assay for mammalian cells (Invitrogen™ L3224). All studies on cell cultures were carried out in 2D and 3D (spheroid) models.

Results and Discussion
CaP nanoparticles had spherical morphology with an average size of 150 nm and a positively charged surface with an average zeta potential of +25 mV. Transfection and gene silencing in human cells with the use of nucleic acid-loaded CaP nanoparticles were demonstrated in both 2D and 3D cell culture models, as well as the uptake of CaP nanoparticles by cells. The nanoparticles were not cytotoxic.

Conclusions
CaP nanoparticles can be successfully used as tools for gene delivery and silencing, both in 2D and 3D cell cultures. They have the potential to be widely applied in the treatment of genetic diseases.

References
[1] V. Sokolova, M. Epple, Angew. Chem. Int. Ed. 47 (2008) 1382-1395.
[2] S. Chernousova, M. Epple, Gene Ther. 24 (2017) 282-289.
[3] M. Zanoni, F. Piccinini *et al.,* Sci. Rep. 6 (2016) 19103.
[4] D. Kozlova, S. Chernousova *et al.,* J. Mater. Chem. 22 (2012) 396-404.

SYNTHESIS AND CHARACTERISATION OF ALGINATE MICRO- AND NANOSPHERES LOADED WITH BOVINE SERUM ALBUMIN

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[ENGINEERING OF BIOMATERIALS 143 (2017) 35]

Introduction

The development of new drug delivery systems (DDS) is improving the efficacy of controlled and targeted drug release systems. DDS are supposed to improve effectiveness of therapy and lessen the harmful side effects of drugs on organisms [1-3]. The preferred drug administration route is orally. Thanks to the composition of mucus and pH varied in different parts of the food track, scientists proposed biopolymers as potential materials for oral drug delivery. Among them, the most interesting one is sodium alginate (SA), a biocompatible and biodegradable polianionic polymer of non-animal origin, which shrinks at pH below 4 [2]. The aim of this study was to obtain alginate particles in two different size regimes with controlled particle-size distribution: nano (<100 nm) and micro (from 100 nm to couple of μm).

Materials and Methods

Sodium alginate spheres were obtained by double emulsion solvent evaporation technique with an external gelation mechanism. Double emulsion was formed using a sonicator probe. To prove the possible application of those particles as new DDS, bovine serum albumin (BSA) was selected as a model drug. The influence of two different types of surfactant was examined (anionic and non-ionic). After the optimisation, four different protocols of synthesis were created – two with sodium cholate (anionic surfactant) and two with Tween80 (non-ionic surfactant).

In brief, 3.5% (v/w) solution of SA, dichloromethane and a proper surfactant (1% Tween80 or 6% sodium cholate) were mixed together. Then, a second volume of surfactant was added to form a double emulsion. Finally, calcium chloride as a cross-linker was added dropwise with stirring. The double emulsion formed was stirred at 700 rpm for 6h in 30°C. Afterwards, the solution was centrifuged with addition of 0.01M $CaCl_2$. Efficiency of BSA encapsulation was calculated based on UV-vis spectroscopy according to BCA Protein Assay (Thermo Sccientific).

Results and Discussion

Nanospheres

Particles of spherical shape with an average size of 27.1 ± 8.2 nm were obtained using sodium cholate as an anionic surfactant (FIG. 1). Those particle-size distributions were confirmed by SEM and DLS analysis. Hydrodynamic radius of 192 ± 5 nm and ζ potential of -29.0 ± 1.6 mV were obtained. BSA was encapsulated in the polymeric matrix with an average efficiency of 48.6% ± 10.0%. Encapsulation was confirmed by FT-IR and confocal microscopy. Importantly, the average ζ potential changed from the initial -29.0 ± 1.6 mV to-12.6 ± 0.7 mV, which can be explained by charge of protein in pH = 5 (in which measurements were carried) – below isoelectric point charge of protein became positive (for BSA isoelectric point is 5.82), thus it could change ζ potential [4].

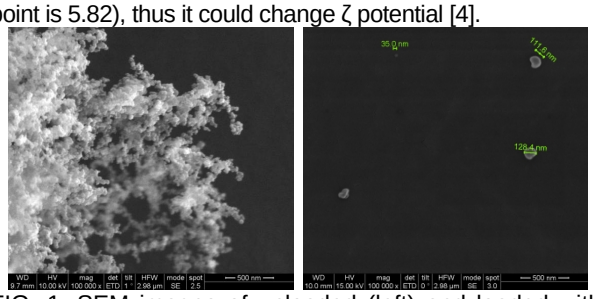

FIG. 1. SEM images of unloaded (left) and loaded with BSA (right) alginate nanospheres. Surfactant: sodium cholate.

Microspheres

Particles of spherical shapes with average size of 0.46 ± 0.11 μm and 0.58 ± 0.21 μm were obtained using Tween80 and lower (0.07% (w/v)) and higher (0.13% (w/v)) concentration of sodium alginate, respectively (FIG. 2). After encapsulation of BSA the increase of size from 0.46 ± 0.11 μm to 0.52 ± 0.13 μm was observed when using the lower concentration of alginate. In the second case (when a higher concentration of SA was used) two distributions were retrieved with sizes around 0.53 ± 0.12 μm and 1.03 ± 0.30 μm.

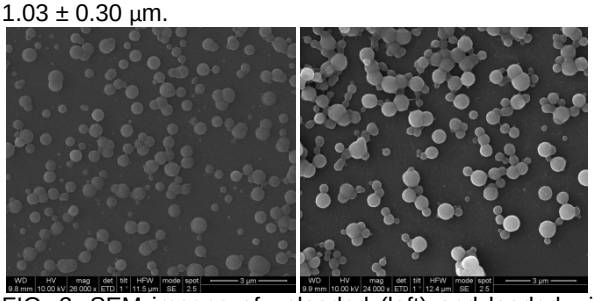

FIG. 2. SEM images of unloaded (left) and loaded with BSA (right) alginate microspheres. Surfactant: Tween80.

As well, it was possible to encapsulate the protein (BSA) in the alginate matrix with average efficiency of 65.71%. Release of protein was carried out in PBS (pH=7.4) at 37°C for 48h showing a release of 8 wt.%. The presence of protein was confirmed by confocal microscopy and FT-IR spectroscopy.

Conclusions

Protocols for the synthesis of alginate particles showing two different size-distribution regimes were successfully optimized. Particles made with Tween80 as a surfactant did not present tendency to aggregation and elevated monodispersity. In addition, particles obtained with the use of sodium cholate as surfactant showed also monodisperse morphologies. BSA was encapsulated in the polymeric matrix with efficiency up to 60%. Release tests revealed that up to 10% of the initial BSA was released after 48h.

Acknowledgments

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References

[1] Y.J. Son, W.J. Kim *et al.*, Arch Pharm Res. 37 (2014) 69-78.
[2] A. Sosnik, ISRN Pharmaceutics (2014) 1-17.
[3] M. Sheikhpour, L. Barani *et al.*, Journal of Controlled Release 253 (2017) 97-109.
[4] E. Bunkute, C. Cummins *et al.*, PIP-DB: the Protein Isoelectric Point database. Bioinformatics 2 (2015) 31.

THE DEPENDENCE OF MECHANICAL PROPERTIES ON TiO_2 NANOPARTICLES CONCENTRATION IN HYBRID HYDROGEL MATERIALS WITH POTENTIAL USE AS BONES SCAFFOLDS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 36]

Introduction

Introduction of TiO_2 to polymer matrices induces the formation of apatites on the surface of the materials, which demonstrates the biological activity of the synthetized hybrids. Such materials can be used as scaffolds for the bone tissue grow. Polymer gelation takes place at about 37°C, which enables the material to be injected into a specific bone defect site and to form the scaffold at the target site.

Materials and Methods

The microstructure of prepared hybrids after mineralization was observed with use of a field emission gun (FEG) scanning electron microscope FEI Europe Company − NOVA NANO SEM 200 equipped with an EDAX energy dispersion spectrometer. The mechanical properties of the tested hydrogels were studied with a Physica MCR-301 (Anton Paar) rheometer equipped with a parallel-plate PP 50 made of stainless steel with a 25 mm diameter. Rheoplus/32 v3.40 software was used. To determine the value of the storage modulus (G') measurements were conducted in oscillation mode using a frequency of 1 Hz and strain of 0.3%, the measuring gap was set at a distance of 0.3 mm. All measurements were performed at 37°C. After cytotoxicity testing the absorbance of the solutions was measured at 450 nm using a microplate reader TECAN Infinite M200.

Results and Discussion

The activity is oriented on optimization of selected TiO_2 nanopowder concentration in the polymeric hybrid materials based on natural polymers (collagen, chitosan) crosslinked with genipine. The introduction of TiO_2 into hydrogel matrices aims to improve mechanical properties and improve the biological activity of materials. The research concerns the development of a material with the highest possible mechanical strength while preserving its biological activity. An anatase TiO_2 nanoparticle with a specific surface area of about 100 m^2/g was used to prepare sample series. Based on previous research, it was found that the addition of TiO_2 to the polymer matrix at 1.9 mg/ml resulted in a deterioration in mechanical properties. Within the project studies with materials with lower TiO_2 concentration were performed as well as cytotoxicity on human osteoblastic bone cells (MG-63) with XTT method and defining the rheological properties of the hybrids (G', G '', viscosity) were carried out. As we already showed in previous publication the addition of TiO_2 to polymer hybrids induces the deposition of apatites on the surface of the materials [1]. This effect was not found for polymer matrices that did not contain TiO_2. There was no significant effect of the polymorphic TiO_2 variation on the properties of the tested systems. Literature reports on other polymer materials with TiO_2 nanoparticles indicate that the addition of oxide, depending on concentration, can affect the mechanical properties by either improving or deteriorating it. This is the reason why it was so important to determine the optimal concentration of TiO_2 that will keep the biological activity without impairing the mechanical properties and may even improve them. The early results suggest that the optimal TiO_2 concentration is within the range of 0.2-0.5 mg/ml

Conclusions

The gained results of the research are the first and fundamental step to solve the scientific problem of obtaining hydrogel hybrid materials with TiO_2 nanoparticles that can be used as bones scaffolds. It was also found that the properties of hybrid materials depend on TiO_2 concentration. The optimal nanoparticle concentration seems to be about six times lower than the one we used in our preliminary research [1] resulting in storage modulus improvement.

References

[1] K. Zazakowny, J. Lewandowska-Łańcucka, J. Mastalska-Popławska, K. Kamiński, A. Kusior, M. Radecka, M. Nowakowska: Biopolymeric hydrogels − nanostructured TiO_2 hybrid materials as potential injectable scaffolds for bone regeneration, Colloids and Surfaces B: Biointerfaces, 148 (2016) 607–614.

INVESTIGATION AND VIZUALIZATION OF THE CELLS GROWN ON CERAMIC COATING BY ELECRTON MICROSCOPY TECHNIQUES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 37]

Introduction
Biocompatibility is a crucial property to be tested for all novel biomaterials. The first step in standard approach is *in vitro* cell culture on the top of tested material and subsequent analysis of the proliferation and viability of the cells after defined time of incubation. Various colorimetric assays together with confocal microscopy are applied to evaluate cells behaviour after contact with biomaterial. In the current work we propose application of focused ion beam-scanning electron microscope (FIB-SEM) tomography as a supplementary technique to typical biocompatibility studies. It enables to investigate the interface between cell and biomaterial at the cross-section [2].

Materials and Methods
Ceramic coating on biomedical titanium alloy Ti6Al7Nb was deposited by micro-arc oxidation (MAO) in the electrolyte containing calcium acetate and sodium phosphate. MG-63 cells were cultured on the top of obtained coatings. Prior to electron microscopy investigation cells were fixed, dehydrated and gold sputtered. Microscope NEON CrossBeam 40EsB (ZEISS) was used to perform FIB-SEM tomography, 3D reconstruction was done by ImageJ 1.44p software. Details concerning coating deposition, cell culture and microscopic investigation are described in Ref. [2].

Results and Discussion
With careful selection of process parameters it is possible to obtain rough and porous coatings containing crystalline hydroxyapatite (HA) in the outer layer by MAO. Elongated crystals of HA, with the length of 300±50 nm and width of 45±10 nm, provided high surface area for cells growth and promoted filopodia formation. MG-63 cells observed with SEM were well spread with the multiple cytoplasmic projections at the surface of the tested coating.
FIB-SEM tomography is the technique that combines cutting of the sample by gallium ions with imaging of exposed surface with secondary or backscattered electrons. Therefore, it is frequently called 'slice-and-view'method [1]. Such approach enables simultaneous sectioning of delicate cell and hard ceramic coating. Collection of about 180 images was used for 3D reconstruction and visualization of the interface between cell and ceramic coating. It presents excellent adaptation of the cell to the rough surface.

Conclusions
Scanning electron microscopy provides an opportunity to investigate cells morphology and behaviour in relation with biomaterial topography. Whereas FIB-SEM tomography allows a detailed investigation of cell-material interactions in the analyzed sample volume.

Acknowledgments
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References
[1] U. Stachewicz, T. Qiao, S.C.F. Rawlinson, F.V. Almeida, W.Q. Li, M Cattell, A H. Barber, Acta Biomater. 27 (2015) 88-100.
[2] J. Karbowniczek, A. Gruszczyńki, A. Kruk, A. Czyrska-Filemonowicz, Acta Phys. Pol., A 131 (2017) 1357-1360.

POLYSACCHARIDES HYDROGEL - RADIATION INDUCED FORMATION AND MEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 38]

Introduction

Permanent hydrogels of synthetic or natural polymers can be formed by chemical methods with utilisation of crosslinking agents. Crosslinkers, typically bi-functional and highly reactive compounds, are often toxic, similarly to initiators or catalysts, thus any traces of such residues may be harmful for the living organism. Moreover, thermal initiation usually excludes possibility of inclusion biologically active molecules due to thermal instability. Radiation method can be employed in order to circumvent those disadvantages, therefore it is especially suitable for manufacturing of hydrogels for biomedical applications. Polysaccharides, despite their general tendency for radiation-induced degradation, can be converted into permanent hydrogels when irradiated with or without additives under certain conditions [1]. The scission of glycosidic bonds is predominantly responsible for the reduction in the molecular weight of macromolecules. However, during irradiation of polysaccharides in an aqueous solution, and when applying specific irradiation conditions, scission and crosslinking take place simultaneously. Whether the outcome is a degraded polymer or a gel is determined by the prevailing mechanism of radicals reactions. Thus, it was demonstrated that polysaccharide derivatives having high molar concentration of substituent side groups prone to create stable carbon-centered radicals, irradiated in highly concentrated solutions or in solutions of specific pH (in the case of ionic polysaccharides) [2]. In this report, a short review of current approaches to crosslink polysaccharides will be reviewed and followed by an exemplary application of carboxymethylchitosan hydrogel for nerve regeneration scaffold.

Materials and Methods

Carboxymethylchitosan (CMCS) of the deacetylation degree (DDA) 93.8%, DS 96% and intrinsic viscosity in 0.1 mol dm^{-3} NaCl of η = 2.77 dm^3 g^{-1} was obtained from Kraeber & Co. GmbH (Germany). Aqueous solutions of CMCS were irradiated by electron beam (EB) with and without a crosslinking agent of poly(ethylene glycol) diacrylate (PEGDA, M_W = 700 g mol^{-1}, Sigma-Aldrich). Obtained gels were evaluated by standard sol-gel analysis. Nerve regeneration conduits were fabricated from a solution of poly(lactic acid) and poly(trimethylene carbonate) mixture by phase-inversion method. LDH and XTT cytotoxicity, and *in vivo* biocompatibility and functional studies were conducted using rat animal model.

Results and Discussion

Results of this study indicated that ionizing radiation is a convenient tool to synthetize hydrogels based on CMCS when irradiated in highly concentrated aqueous solutions. Irradiation of 12% CMCS, as the optimum concentration, leads to formation of hydrogel scaffold at a dose of 25 kGy. Since not all macromolecules form the network, which is due to partial degradation of the polysaccharide, the gel fraction (GF, mass of insoluble fraction per mass of used polymer) was evaluated along with the determination of the equilibrium degree of swelling (EDS, grams of water per gram of gel). The GF and EDS of the gels were ca. 30% and 50 g/g.

Results of LDH and XTT cytotoxicity tests and *in vivo* examination of local tissue response were an indicator of the good biocompatibility of CMCS hydrogel. The CMCS gels were manufactured *in situ* inside the lumen of nerve guidance tube by irradiation of the tube prefilled with CMCS solution/paste, (the physical gel). The internal gel is strong enough to support regenerating nerve, but also its softness will not obstruct the regrowing cone of the nerve to reach its distal part.

Preliminary animal studies, involving discontinued femoral nerve of a rat showed positive results of nerve regeneration. The nerves were reconnected, as observed in histopathological analysis. Number of neuroma occurrence at the reconnection site was reduced as compared to simple ends suturing (end-to-end connection as a control). Functioning of the nerve resulted in gradual return of motoric functions.

Conclusions

The study highlights the potential of carboxymethylchitosan hydrogel as lumen filling of nerve regeneration channel based on biodegradable polymer blends of poly(lactic acid) and poly (trimethylene carbonate). After *in situ* synthesis of the gel inside the tube, the product is ready for immediate use, because applied technology combines gel formation and sterilization into a single process. This can be accomplished if the dose applied for CMCS hydrogel formation is of 25 kGy or more, as in the present studies. Preliminary animal studies demonstrated biological safety of the CMCS hydrogel, and functional evaluation using rat model revealed positive results of regeneration of femoral nerve.

References

[1] Al-Assaf, S.K.H. Gulrez, R. Czechowska-Biskup, R.A. Wach, J.M. Rosiak, P. Ulanski, Radiation modification of polysaccharides; in: *The radiation chemistry of polysaccharides*, Eds: S. Al-Assaf, *et al*. X. Coqueret, K. Mohd Dahlan, M. Sen, P. Ulanski, IAEA, ISBN: 978-92-0-101516-7, Vienna, 2016.

[2] R. Wach, *et al.*, Carbohydrate Polym 112 (2014) 412-415.

THE INFLUENCE OF THE NANOHYDROXYAPATITE AND GELATIN ADDITIVES ON SELECTED PROPERTIES OF PDLG ELECTROSPUN FIBERS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 39]

Introduction

Electrospinning is one of the most effective methods to obtain homogenous fibers with desired dimensions and properties [1]. This technique allows to utilize the solutions made of natural and synthetic polymers for fibrous meshes fabrication. Native proteins like collagen and gelatin improve the wettability and degradation rates of the synthetic material. On the other hand tricalcium phosphate and hydroxyapatite accelerate the regeneration of the bone by improving osteogenic and mechanical properties of the polymeric fibers [2,3]. M. Mehrasa et al. confirmed the influence of the gelatin PLGA and increase of their hydrophilicity [5]. In presented study investigation of the impact of the gelatin and nanohydroxyapatite addition on the improvement of the morphology and selected physical properties of the fibrous meshes was carried out in order to increase the biocompatibility of the PDLG electrospun fibers.

Materials and Methods

D,L-poly(lactic-co-glycolic acid) (PDLG) with 50:50 lactide to glycolide ratio was purchased from Corbion Purac, Netherlands. The solvent, 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was obtained from Fluorochem, UK. Nanohydroxyapatite and Gelatin (G) type A from porcine skin were obtained from Sigma-Aldrich. Electrospun solution was prepared by dissolving PDLG (PD) in HFP with 10% (w/v) concentration. The mixture was stirred vigorously for 24h. Nanohydroxyapatite (H) was added to the polymer solution with the weight ratios of PD to H of 95:5, 90:10 and 85:15, respectively. Gelatin was used initially in two concentrations within the solutions, with ratios of PD to G of 90:10 and 93:7. However, preliminary trials of electrospinning of mentioned solutions demonstrated the applicability for further experiments only the solutions with PD to G ratio of 93:7. Therefore this content of gelatin within composites was used in further studies. Similar, optimization of the hydroxyapatite content was carried out to obtain the fibers without particles agglomerates. Based on process conditions and SEM observations 10% content (w/w) within the composite for hydroxyapatite was selected. The fibrous substrate was formed via electrospinning method, when a high voltage of 7 kV was applied to the solutions. A collector covered with aluminum foil located at 15 cm distance from the tip of the needle was used. The flow rate was set at 1.0 mL/h. The electrospun fibers were dried in the drying oven for a few days. The Scanning Electron Microscope (SEM) was used to observe the morphology and to estimate the diameters of the obtained fibers. In order to investigate the wettability of the fibrous meshes the water contact angle measurements were conducted. To identify the chemical composition of the surface of obtained fibrous structures Fourier Transform Infrared (FTIR) Spectroscopy was applied. Finally, to determine the degradation rate of the particular types of samples electrospun scaffolds were immersed from 7 up to 30 days in Phosphate Buffer Solution and incubated in 37^{o}C.

Results and Discussion

The SEM images show the morphology of the PD, PDG, PDH and PDGH fibers (FIG. 1). The pure polymeric fibers have smoother surface but larger diameter compared to composite ones. A significant decrease of the fibers diameter from 1198 ±152 nm to 344 ±59 nm was observed along with an increase of the mass fraction of the gelatin and nanohydroxyapatite.

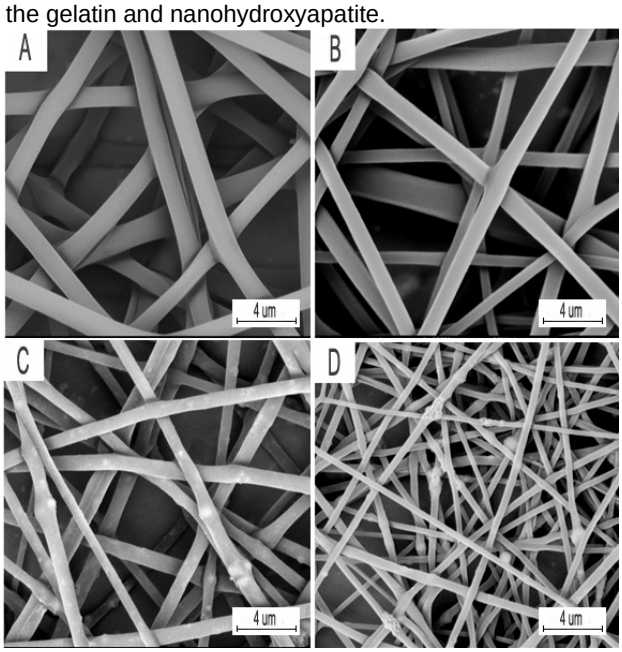

FIG. 1. SEM images of: (A) PD, (B) PDG 93:7, (C) PH 90:10 and (D) PDGH 83:7:10 fibers.

FTIR analysis of the composite fibers revealed peaks, which are characteristic for gelatin and hydroxyapatite. The wettability measurements showed a hydrophobic nature for the PDLG fibers. The gelatin addition caused an increase of hydrophilicity of the surface of polymeric fibers. The values of the contact angle were reduced from 131.74° ±2.25° for PDLG to 73.08° ±7.70° for PG, 126.19° ±1.06° for PH and 55.41° ±5.50° for PGH fibers. Mass loss during degradation was higher for the fibers containing gelatin than for the pure polymeric and polymeric/ceramic fibers. It is probably related to the solubility of the gelatin in aqueous solutions.

Conclusions

According to obtained results, modification of the PD fibers with gelatin and nanohydroxyapatite additives have a great influence in order to receive nanoscale fibers with improved biocompatibility and composite PDGH fibrous meshes are promising substrate in tissue engineering applications.

Acknowledgments

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References

[1] Kijeńska, E. et al. Chapter 2. In: T. Uyar and E. Kny, Electrospun Materials for Tissue Engineering and Biomedical Applications, 2017, 1st ed. pp.43-56.
[2] D. W. Hutmacher, Biomaterials. 21 (2000) 2529–2543.
[3] E. Kijeńska et al. Int J Polym Mater 2016, 65 (16): 807-815.
[4] M. Mehrasa, M. A. Asadollahi, et al., Int. J. Biol. Macromol. 79 (2015) 687–695.

FABRICATION OF CUSTOM DESIGNED SPINAL DISC REPLACEMENT FOR VETERINARY APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 40]

Introduction

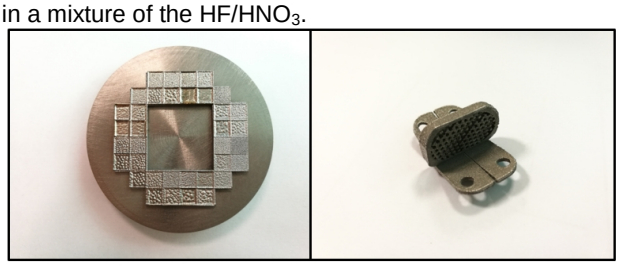
Two-phase alpha-beta titanium alloy Ti-6Al-7Nb is widely used in many industrial applications. Due to excellent biocompatibility and non-toxicity in human body environment and excellent mechanical properties it is an attractive material in medical field. Difficulties with fabrication complex shaped medical implants and gradient or lattice structures from Ti-6Al7Nb alloy lead to finding out fabrication method which allows to produce such elements. The most appropriate for mentioned applications are Additive Manufacturing (AM) techniques such as Selective Laser Melting (SLM), which enables producing any geometry directly from the Computer Aided Design (CAD) model [1]. The aim of the study was fabrication of custom designed spinal disc replacement for veterinary applications.

Materials and Methods

In our study both cuboid specimens and spinal disc replacement, were fabricated using Realizer SLM50 machine. The SLM50 is a 3D printer for metals and its alloys equipped with Nd:YAG laser with maximum power of 120 W. Cuboid shaped samples of 6x6x3mm size were fabricated with the energy densities in range from 38 to 333 J/mm^3 and scanning speeds from 125 to 375 mm/s to select the best manufacturing parameters of the Ti6Al7Nb alloy. The process was conducted in argon atmosphere. Afterwards, samples were cut perpendicularly to the platform surface. Metallography cross section related to x-z scanning plane were mechanically gridded with SiC papers with gradation from 320 to 1200 µm and polished with 0.1 µm Al_2O_3 suspension. Etching was performed with HF, HNO_3 and H_2O mixture [2]. Microstructure observation was performed using Zeiss Axio Light Microscope. Microhardness measurement was carried out on Zwick/Roell machine with load of 1.961 N. Finally, spinal disc replacement designed using a computed tomography, basing on the case of a clinical veterinary patient, was fabricated using selected manufacturing parameters. After ultrasonic cleaning in water, chemical polishing in HF/HNO_3 solution was performed to improve surface quality of the implant and remove unmelted fully powder particles from struts. Furthermore, it was confirmed in our previous study that treating titanium with solution of HF/HNO_3 has positive influence on cell response [3].

Results and Discussion

Different manufacturing parameters used for each sample fabrication had influence on their surface quality (FIG. 1a). For some samples irregular or concave surfaces were observed while for others smooth with very regular boarding. Microscopic observations showed that porosity was also determined by the manufacturing parameters. The best surface quality and the lowest porosity of about 99.7% was obtained for sample manufactured with the energy density of 50 J/mm^3 and the scan speed of 375 mm/s. The average microhardness of this sample was 368 ±12 $HV_{0,2}$ and was 26% higher than for pure titanium (CP Ti) fabricated by SLM technique [4]. Spinal disc replacement implant was fabricated using the manufacturing parameters adopted from the cubic sample (FIG. 1b). Our optimization procedure provided the high manufacturing accuracy of the CAD model. Furthermore, surface quality of the spinal disc replacement was improved after chemical polishing in a mixture of the HF/HNO_3.



(a) (b)

FIG. 1. (a) Samples produced with different manufacturing parameters; (b) Spinal disc replacement.

Conclusions

The microstructure and the mechanical properties of Ti-6Al-7Nb produced by SLM technique is determined mainly by delivered amount of energy density (J/mm^3) and scan speed value (mm/s). Selecting proper manufacturing parameters is important to obtain high manufacturing accuracy of the CAD model. Furthermore, proper chemical polishing procedure improves surface quality of elements produced by SLM technique for medical application.

Acknowledgments

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References

[1] A. Pawlak, P. Szymczyk, G. Ziolkowski, E. Chlebus, B. Dybala, Fabrication of microscaffolds from Ti-6Al-7Nb alloy by SLM, Rapid Prototyp. J. 21 (2015) 393–401. doi:10.1108/RPJ-10-2013-0101.
[2] E. Lyczkowska, P. Szymczyk, B. Dybała, E. Chlebus, Chemical polishing of scaffolds made of Ti-6Al-7Nb alloy by additive manufacturing, Arch. Civ. Mech. Eng. 14 (2014) 586–594. doi:10.1016/j.acme.2014.03.001.
[3] B. Wysocki, J. Idaszek, K. Szlązak, K. Strzelczyk, T. Brynk, K. Kurzydłowski, W. Święszkowski, Post Processing and Biological Evaluation of the Titanium Scaffolds for Bone Tissue Engineering, Materials (Basel). 9 (2016) 197. doi:10.3390/ma9030197.
[4] B. Wysocki, P. Maj, A. Krawczyńska, K. Rożniatowski, J. Zdunek, K.J. Kurzydłowski, W. Święszkowski, Microstructure and mechanical properties investigation of CP titanium processed by selective laser melting (SLM), J. Mater. Process. Technol. 241 (2017) 13–23. doi:10.1016/j.jmatprotec.2016.10.022.

HYBRID, BIOACTIVE COATINGS FORMED ON TITANIUM ALLOYS SURFACE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 41]

Introduction

Plasma electrolytic oxidation (PEO) is one of widely used electrochemical methods for light metal surface functionalization [1-3]. Porous oxide layer might be composed by desirable, bioactive compounds incorporated from anodizing bath. However, the PEO method is limited to incorporation of compounds which are sensitive for solvents and spark discharges occur during the process. Especially drugs or vitamins cannot be incorporated into oxide layer using this technique. However, on the porous oxide layer these biologically active substances can be deposited with layer of degradable polymer.

One of the β-phase titanium alloy Ti-15Mo is considered as a promising material for bone implants. This titanium alloy is composed only of biocompatible elements, and exhibits low Young's modulus (~ 60 GPa) [4-6]. It is very easy to modify surface of the Ti-15Mo alloy by bioactive oxide layer. To provide antibacterial and therapeutic properties to the outer surface, drugs like doxycycline, cephalosporins (e.g. III or IV generation) or amoxicilline might be blended with polymer. These kinds of hybrid layers might be used especially for dental implants.

Materials and Methods

Surface of Ti-15Mo alloy was modified by plasma electrolytic oxidation process. The PEO process was carried out in solution composed of $Ca(H_2PO_2)_2$ and $Ca_3(PO_4)_2$. Applied voltage during the process was 300 V, when current density was 100 mA/cm^2. On the porous oxide layer the fast-degradable polymer (poly(D,L-lactide-co-glycolide - PLGA) was deposited using dip coating method. To obtain antibacterial properties of the surface, the polymer was blended with doxycycline (5% w/v polymer). Hybrid, oxide-polymer coatings were characterized using scanning electron microscope (SEM, Phenom ProX), Raman spectroscopy with CDD detector. Degradation of polymer layer and drug release was carried out in artificial saliva up to 4 weeks. Changes in polymer chain structure were monitored using ^{1}H NMR, when amount of released drug was determined using HPLC technique. Cytocompatibillity of the layers was evaluated using osteoblast-like MG-63 cells. Cell metabolic activity was evaluated using Alamar Blue reagent. The viability, attachment and distribution of the adhered cells were evaluated using live/dead staining.

Results and Discussion

FIG. 1. presents SEM image of the PLGA layer deposited on the porous oxide layer formed on Ti-15Mo alloy surface using PEO method. The polymer layer covered the oxide layer, however some characteristic structure of porous layer was still visible. Results from ^{1}H NMR confirmed that PLGA was degraded up to 4 weeks of immersion in artificial saliva at 37°C. Raman spectroscopy confirmed presence of polymer layer with and doxycycline. HPLC measurements confirmed that doxycycline was released within the first one hour during hybrid layer immersion in artificial saliva at 37°C.

Cytocompatibillity investigations showed that all of the layers were not cytotoxic. After 1 day of culture the number of cell increased on all of the investigated samples. The highest percentage of Alamar Blue reduction was for the sample with polymer layer without drugs. However, the experiment showed that the layer with doxycycline were slightly less cytocompatible. On all modified surfaces cells were well adhearing and small amount of dead cells was observed. After 3 and 7 days of culture, the samples with polymer layers exhibited better cytocompatibility than reference sample (unmodified Ti alloy) and only anodized sample.

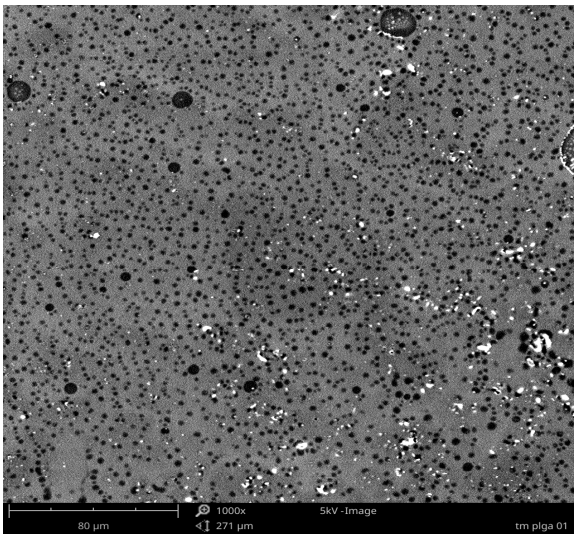

FIG. 1. SEM image of hybrid oxide-ceramic coatings formed on Ti-15Mo alloy surface. Magnification: x1000.

Conclusions

Surface of the Ti-15Mo alloy was functionalized in order to provide it bioactive and antibacterial properties. Doxycycline was released from the polymer layer in a very short time. Hybrid layers were found cytocompatible with the osteoblast-like MG-63 cell up to 7 days of culture. Next steps of the hybrid layer characterization will contain advanced biological experiments with mesenchymal steam cells and selected gram positive and gram negative bacteria.

Acknowledgments

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References

[1] A. Gupta, M. Dhanraj, G. Sivagami, Indian J. Dent. Res. 21 (2010) 433-438.
[2] K. Rokosz, T. Hryniewicz, *et al.,* Mater. Sci. Forum Vol. 862 (2016) 86-95.
[3] A.R. Rafieerad, M.R. Ashra *et al., Mater*. Sci. Eng. C 57 (2015) 397–413.
[4] S. Radin, P. Ducheyne, J. Biomed. Mater. Res. 30 (1996) 273–279.
[5] W. Ji, F. Yang *et al.,* Biomaterials 33 (2012) 6604–6614.
[6] M. Sampath, R. Lakra et al., Colloids Surf. B: Biointerfaces 117 (2014) 128–134.

SELF-GELLING, INJECTABLE HYDROGEL-BIOACTIVE GLASS COMPOSITES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 42]

Introduction

When applying hydrogels as biomaterials for bone tissue regeneration, the introduction of an inorganic phase is considered desirable [1]. Particles of bioactive glass can be added during hydrogel formation. Bioactive glasses are not only known to promote bioactivity, but they can also induce the gelation of solutions of anionic, calcium-binding polymers and impart antibacterial activity [2]. Bioactive glasses can be doped with metal ions such as magnesium (Mg), zinc (Zn) and strontium (Sr) [3].
In this study, four different bioactive glass preparations were added to a solution of pectin, a calcium-binding polysaccharide which gels in the presence of calcium. The resulting composites where characterized physicochemically (gelation kinetics), microbiologically and cell biologically with MG63 osteoblast-like cells.

Materials and Methods

Bioactive glasses, doped with Zn, Mg and Sr and undoped, hereafter denoted P5 (undoped), P5-Zn, P5-VS-Mg and P5-VS-Sr were produced as described previously [3]. Glass particle sizes were studied by laser diffraction: average (d(0,5)) particle sizes were 31, 24, 91 and 92 µm, respectively. Glasses and pectin solution (amidated apple pectin, 0.8% (w/v)) were sterilized by autoclaving at 134ºC. Hydrogel-glass composites were prepared by vigorous mixing of glass particles with pectin solution to yield composites with glass concentration of 32% (w/v). Visually, the distribution of glass particles appeared to be more homogeneous in composites containing P5 and P5-Zn. Gelation kinetics were studied by rheometry. To assess the cytocompatibility of hydrogel-glass composites, 50 µl freshly prepared composite was dispensed into wells of a 96 well plate. Following gelation, MG63 osteosarcoma cells were seeded on top of the thin composite layer at 10,000 ($1X10^4$) cells per well in complete growth medium (Dulbeccos Modified Essential Medium, 10% Foetal Bovine Serum, 1% PenStrep). Cells were incubated at 37°C, with 5% CO_2 until required. After 1, 3 and 7 days, cell viability was assessed using the alamarBlue® assay (Thermo Fisher; DAL1025). AlamarBlue metabolic activity readings were normalised to non-seeded gels in complete growth medium to account for any effect this may have had on the resazurin reduction. Cells seeded on tissue culture plastic were used as a control to assess proliferation potentials. MG63 cells were also encapsulated into composites containing P5 and P5-Zn (10,000 cells/50 µl composite), as were the most homogeneous and easiest to handle. Antibacterial activity was tested using methicillin-resistant *Staphylococcus aureus* (MRSA) as described previously [4] and *Staphylococcus aureus (S.aureus).*

Results and Discussion

Gelation of all composites occurred within 5 minutes. Composites did not exhibit any appreciable antibacterial activity against MRSA, but composites containing P5-Zn inhibited *S.aureus* growth. MG63 cells retained viability and proliferated over 7 days on composites containing P5 and P5-VS-Sr (FIG. 1). Proliferation was markedly lower on composites containing P5-VS-Mg and P5-Zn at all time points. This is surprising, considering that Mg, as a component of ceramic materials, is able to stimulate cell proliferation [5]. MG63 cells also retained viability when encapsulated in composites containing P5 and P5-Zn (FIG. 2). After encapsulation, proliferation was markedly higher in composites containing P5-Zn than in those containing P5 after 3 and 7 days, although the proliferation values on composites containing P5-Zn were markedly lower (FIG. 1). The reasons remain unclear.

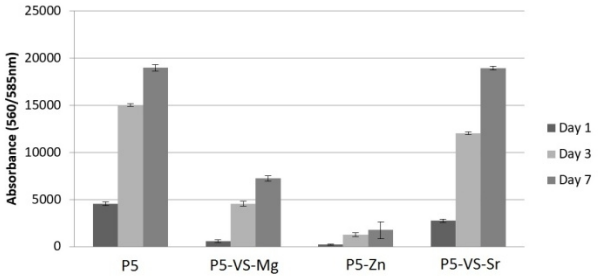

FIG. 1. MG63 cell proliferation on the surface of composites after 1, 3 and 7 d.

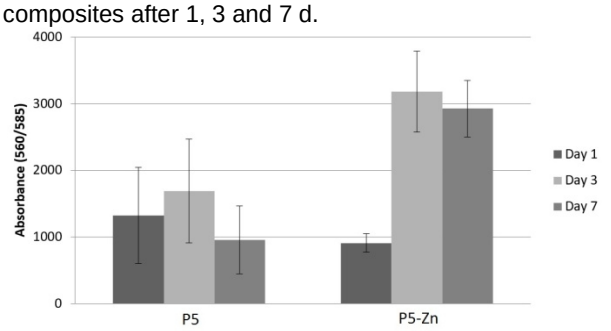

FIG. 2. Proliferation of MG63 cells encapsulated in composites after 1, 3 and 7 d.

Conclusions and Outlook

All four bioactive glass preparations induced gelation of pectin solution to form injectable hydrogel-bioactive glass composites within 5 minutes. Osteoblast-like MG63 cells were able to maintain viability and proliferate both on the surface of composites and after encapsulation. Glass distribution homogeneity and cell number depended on type of glass used. These results pave the way for further investigation of mineralizability and glass distribution.

Acknowledgments

Petra Rigole is thanked for technical assistance.

References

[1] Gkioni et al, Tissue Eng Part B Rev 2010,16,577
[2] Douglas et al, Biomed Mater. 2014, 9. 045014
[3] Ciołek et al, Prace ICiMB 2014, 16:17
[4] Douglas et al, Biomed Mater. 2016, 11, 045015
[5] Douglas et al, J Tissue Eng Regen Med 2016,10,938.

PECTIN COATINGS ON TITANIUM ALLOY SAMPLES PRODUCED BY ADDITIVE MANUFACTURING: PROMOTION OF HUMAN BONE MARROW STROMAL CELL PROLIFERATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 43]

Introduction

Titanium and its alloy, e.g. Ti6Al4V, are popular load-bearing biomaterials for bone contact. They can be fabricated by additive manufacturing technologies. The adhesion and proliferation of bone-forming cells is a pre-requisite for formation of new bone tissue on the implant surface, which in turn leads to implant stability and long-term success. Hence, surface coatings which promote cell adhesion and proliferation are desirable.
In this study, Ti6Al4V discs prepared by additive manufacturing were coated with layers of pectins, calcium-binding polysaccharides derived from citrus (C) and apple (A) containing alkaline phosphatase (ALP), the enzyme responsible for mineralization of bone tissue. Coatings were characterized biologically with human bone marrow stromal cells (hBMSC). Cell adhesion and proliferation were assessed.

Materials and Methods

Rough Ti6Al4V discs of diameter 2 cm were prepared as described previously [1]. Ti6Al4V discs and 0.8% (w/w) C (degree of esterification (DE) 34%, Galacturonic acid content (GalC) 74%) and A (DE 35%, GalC 75%) pectin solutions were autoclaved at 134ºC. ALP solution (1.6% (w/v)) was sterilized by filtration. ALP and pectin solutions were mixed 1:1 (v/v). 250 µl of this solution was spread on Ti6Al4V and allowed to air-dry in a laminar flow bench. The presence of a coating was confirmed by SEM after gold coating. hBMSC from two different donors were seeded at a density of 7,000 cells/cm². Cells were seeded onto the samples in 400 µl of cell culture medium (DMEM with 10% heat-inactivated fetal calf serum, and antibiotics (penicillin and streptomycin). After 2 h the medium was filled up to 4 ml and culture proceeded at 37°C in a humified CO_2 incubator. Proliferation was assessed by the MTS-Assay. Cells were treated with 10% dye solution in DMEM for 2 h. Analyses were performed 24 h and 7 days after seeding. Statistical significance was analyzed by one-way ANOVA and Bonferroni post-test (prism graph pad software). Cell morphology was assessed after 24 h. Cells were fixed with 4% paraformaldehyde and stained with Alexa488-phalloidine to visualize F-actin cytoskeleton (green fluorescence) and with DAPI to stain the nuclei (blue fluorescence). The images (three from each sample) were taken with Axiophot microscope (Zeiss) using a digital camera and Axiovision software. Focusing of cells of samples was complicated by the roughness of the sample surfaces.

Results and Discussion

A-ALP and C-ALP coatings formed on Ti6Al4V discs. Cells retained viability and proliferated over 24 h and 7 days (FIG. 1). Proliferation was significantly higher on C-ALP coatings than on A-ALP coatings after 1 day, and after 7 days, higher than on both uncoated samples and A-ALP coatings. A-ALP coatings were significantly superior to uncoated samples after 7 days. The reasons for this remain unclear. Cells on all substrates displayed a spread morphology and distinct, well organized F-actin fibers, characteristic for good adhesion (FIG. 2).

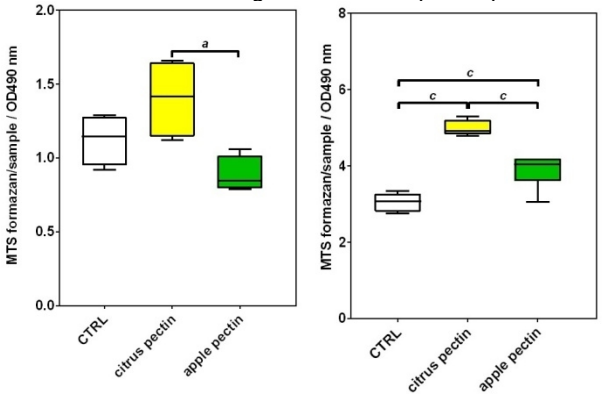

FIG. 1. MTS assay 24 h (left) and 7 days (right) after seeding of hBMSC on samples, a and c indicate significant differences (a: $p<0.05$, c: $p<0.001$).

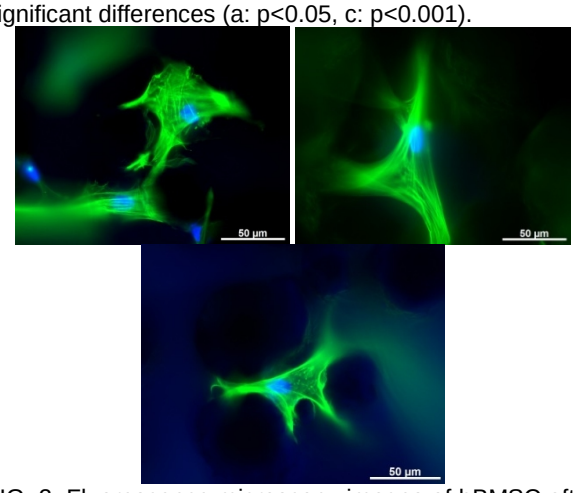

FIG. 2. Fluorescence microscopy images of hBMSC after 24 h. Top left: uncoated Ti6Al4V. Top right: C-ALP coating. Bottom: A-ALP coating. Blue: cell nucleus. Green: F-actin fibres. Scale bar: 50 µm.

Conclusions and Outlook

hBMSC proliferation after 7 days was increased by A-ALP coatings and, in particular, by C-ALP coatings. Cell morphology was similar on coated and uncoated samples. Future work should focus on differentiation.

Acknowledgment

Era-Net Rus Plus program for financial support in the framework of the project “Fabrication and investigation of new hybrid scaffolds with the controlled porous hierarchy for bone tissue engineering” (Intelbiocomp).

References

[1] Surmeneva MA et al, IOP Conf. Series: Materials Science and Engineering 98 (2015) 012025.

DEVELOPMENT OF THERMOSENSITIVE HYDROGELS OF CHITOSAN, SODIUM AND MAGNEISUM GLYCEROPHOSPHATE FOR BONE REGENERATION APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 44]

Introduction

Thermosensitive injectable hydrogels based on chitosan neutralized with sodium beta-glycerophosphate (Na-β-GP) have been studied as biomaterials for drug delivery and tissue regeneration [1]. Magnesium (Mg) has been reported to stimulate adhesion and proliferation of bone forming cells [2]. With the aim of improving the suitability of the aforementioned chitosan hydrogels as materials for bone regeneration, Mg was incorporated by partial substitution of Na-β-GP with magnesium glycerophosphate (Mg-GP). Chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels were also loaded with the enzyme alkaline phosphatase (ALP) which induces hydrogel mineralization [3].

Materials and Methods

4 mL chitosan solution (25 mg/mL in 0.1 M HCl,) 0.4 mL Na-β-GP (1 g/mL Milli-Q water) or Na-β-GP/Mg-GP (0.9 g Na-β-GP and 0.09 g Mg-GP/mL Milli-Q water) solution-suspension, and 0.4 mL ALP solution (25 mg/mL in MilliQ-water) were mixed together to yield 4.4 mL hydrogels. Gelation took place at 37°C overnight.
Hydrogel gelation kinetics was studied by rheometry. Hydrogel mineralization was assessed by incubation in simulated body fluid (SBF) for 14 d, followed by drying and FTIR, TEM and SAED. Hydrogels were characterized biologically by cultivating MG63 osteoblast-like cells on hydrogels and performing a Live/Dead assay. MG63 cells were also cultivated in eluates from hydrogels and growth was assessed using the MTT test.

Results and Discussion

Substitution of Na-β-GP with Mg-GP did not negatively influence gelation kinetics (FIG. 1). Crystalline deposits were observed in both chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels after incubation in SBF (FIG. 2). Cell biological testing showed that both chitosan/Na-β-GP and chitosan/Na-β-GP/Mg-GP hydrogels were cytocompatible towards MG63 osteoblast-like cells (FIG. 3).

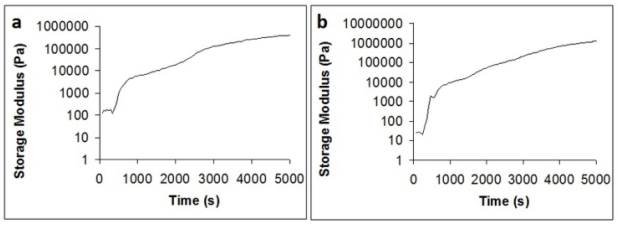

FIG. 1. gelation kinetics of chitosan/Na-β-GP (a) and chitosan/Na-β-GP/Mg-GP (b) hydrogels.

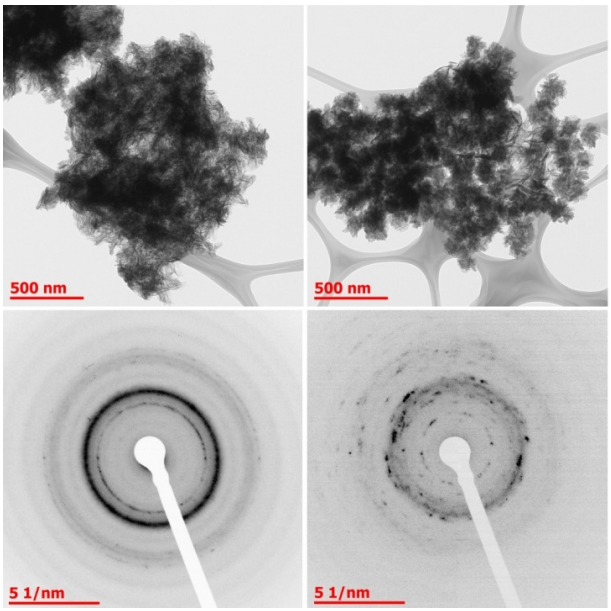

FIG. 2. TEM and SAED of chitosan/Na-β-GP (left) and chitosan/Na-β-GP/Mg-GP (right) hydrogels after incubation in SBF for 14 days.

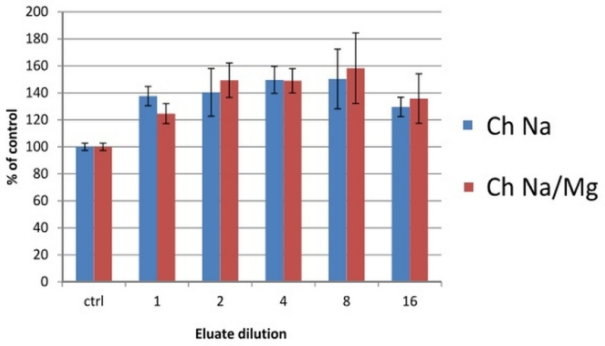

FIG. 3. Growth of MG63 cells in eluate from chitosan/Na-β-GP (Ch Na) hydrogels (blue) and chitosan/Na-β-GP/Mg-GP (Ch Na) hydrogels (red).

Conclusions

Chitosan/Na-β-GP/Mg-GP hydrogels can be used as an alternative to chitosan/Na-β-GP hydrogels for bone regeneration applications. However the incorporation of Mg in the hydrogels during hydrogel formation did not bring any physicochemical or biological benefit.

Acknowledgments

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References

[1] Chenite *et al.* Biomaterials 2000, 2155–61.
[2] Douglas *et al.* J Tissue Eng Regen Med 2016,938-54
[3] Douglas *et al.* Int J Biol Macromol, 2013, 122–32.

HIGH-TEMPERATURE MICROSCOPY: A PRACTICAL TOOL FOR BIOCERAMICS COMPOSITES EXAMINATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 45]

Introduction

High-temperature microscopy (HTM) is a great characterization technique, that allows to investigate the thermal behavior of material under the heating. This method is appropriate for examinations of glasses, slags, ceramics, glazes and various of raw materials by *real-time* observe and record (photo capture or video record) sample contours changes with temperature increase. Results of HTM provides valuable information of the characteristic temperatures of material (i.e. sintering softening, melting and flowing) and also viscosity, wettability and surface tension and also can be very useful for biomaterials characterization [1-3].

Materials and Methods

In this work HTM was used to evaluate the ceramics biocomposites modified with two kinds of sol-gel bioactive glasses (SBG) addition. The SBG glasses were from the SiO_2-CaO-P_2O_5 system with high CaO or high SiO_2 content. The composites were fabricated with different SBG content combined with TCP (Tricalcium phosphate), HA (Hydroxyapatite) as well as titanium dioxide (TiO_2). The experiment was proceed until temperature reached 1400□C or sample was completely melted. The HTM examinations were performed in order to estimate the characteristic temperatures of prepared samples i.e. sintering, softening, melting and flowing.

Results and Discussion

Results of conducted high-temperature microscopy examinations showed that for all types of composites it was possible to estimate the sintering temperatures. For composites modified with high CaO content SBG also melting and flowing temperatures were pointed. Obtained data were used to established the sintering curves of the materials. The differences in characteristic temperatures were mostly caused by the chemical composition of the bioactive glasses used as composites addition. Generally the glass with high SiO_2 content did not significantly influenced the thermal behaviour of bioceramics composites. Meanwhile addition of high CaO content glass resulted in lower sintering and softening temperatures in comparison with raw HA, TCP and TiO_2 materials as well as melting and flowing occurred.

Conclusions

In order to obtain ceramics biocomposites it is necessary to prosecute sintering process in adequate temperatures, in this work we demonstrated that High-Temperature Microscopy is perfect method for this assignment.

Acknowledgments

This study was carried out within the AGH University of Science and Technology (Krakow), Grants Number: 11.11.160.365 and 15.11.160.203(B3).

References

[1] Cahn R.W., Haasen P., Cramer E.J., "Materials Science and Technology, Vol. 2B: Characterisation of Materials", Wiley-VCH, 1993

[2] Shelby, J.E., Introduction to Glass Science and Technology (2nd ed.). The Royal Society of Chemistry, Cambridge, 2005

[3] Amelinckx S., van Dyck D., van Landuyt J., van Tendeloo G., "Handbook of Microscopy, Applications in Materials Science, Solid-State Physics and Chemistry", VCH, Weinheim, 1997

OPERATIVE TREATMENT OF BONE FRACTURE WITH KIRSCHNER WIRES – YOUNG CAT CASE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 46]

Introduction

Cats, despite having natural abilities of agile moving and absorbing jumps from heights sometimes suffer injuries. They occur in a consequence of entangle of their leg, being hit by a car or attacked by other animal or human. Injured cat requires veterinary treatment.
At first, depending on kind of injury, it's recommended to give some medicine against shock and general painkillers which should calm the animal and prepare for further treatment. Next radiological examination should be performed in order to define injuries and help in decision of further steps and prevision concerning wound healing. The technique of anastomosis with the usage of nail or wire is well known and often used to help small animals [1-4].
This case study shows technique of joining cats hulled bone base of further radial bone and the ulna using Kirschner wires.

Materials and Methods

European cat, age 10 months, delivered to the ARKA treatment center with the suspicion of sprain joint, around ulna, in the front leg. The RTG diagnose proved a few day hulled bone base of further radial bone and styloid process of the ulna. Such an injury is typical for young cats with immature skeleton and endless growing process. Such an injury is very often treated by proper adjusting of broken leg and application of stiffening bandage. Kirschner wires have been proposed as the most effective method of treatment for this case. Additionally proposed wires might not only sustain weight of the animal but also ensure stable position of the leg. Cat has been sedated and then put into inhaled narcosis. The leg has been prepared to the surgery by disinfection of area around broken part, removal of fur and skin layers. Afterwards bone has been properly placed and stiffed with two Kirschner wires framed in type X shape going through the base further and cranium radial bone. After one month RTG analysis have been performed, and Kirschner wires have been removed during reoperation. The wires have been observed using electron microscope equipped with X-ray energy dispersion spectrometer.

Results and Discussion

Methods of inner anastomosis of cats bones is very comfortable as they allow quite normal behavior of the animal during the convalescence. There is just a little wound outside, with little stiches. Cat straight after narcosis is able to walk what also shortens time required for healing. Of course cat should have limited area to freely move, number of jumps should be minimized.
FIG. 1 shows bone with Kirschner wires after implantation. It was proved that this kind of fixation was done properly. There was a complete healing process observed.

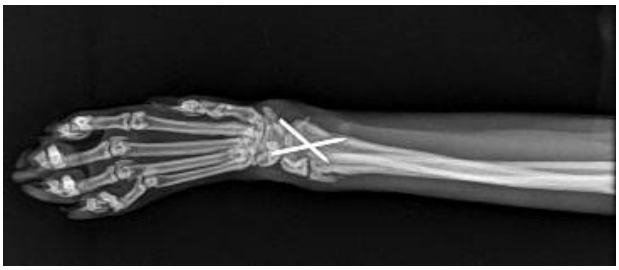

FIG. 1. RTG of bone with Kirschner wires after one month of implantation.

FIG. 2 shows microstructure of wires just after removing them from the leg. Examined surface is smooth, with no signs of corrosion. The EDS analysis confirmed chemical composition of examined wires. Some minor tissues have been also noticed on the investigated surface. In the tissue area nickel was not present what is important due to the irritant properties of nickel.

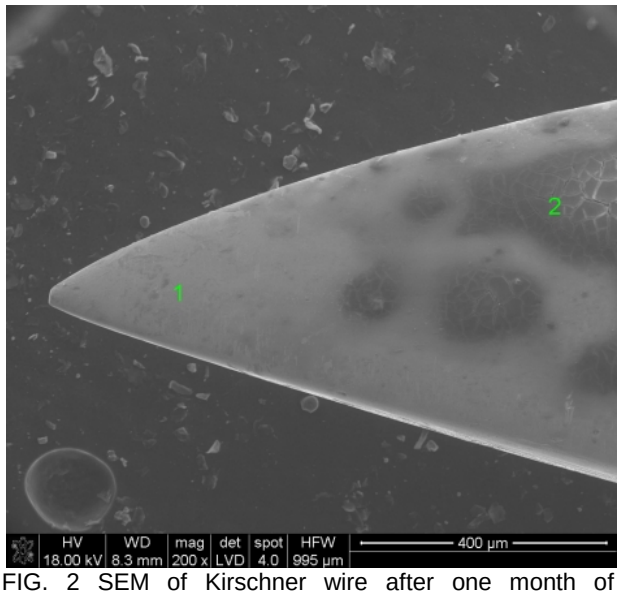

FIG. 2 SEM of Kirschner wire after one month of implantation.

Conclusions

After 4 weeks from anastomosis the leg showed proper functionality. No inflammation has been noticed. Surgery treatment with the usage of Kirschner wires is one of the most effective techniques of healing broken ulna especially at young animals.
Combination of scanning electron microscopy and energy dispersive x-ray spectroscopy is a useful method for investigation corrosion on extracted metallic implants. Both techniques are effective in tracking changes in chemical composition during degradation or corrosion of implants.

Acknowledgments

Authors would like to thank all personnel from ARKA for the care of a sick cat, named Ivan.
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References

[1] A.R. Coughlan, A. Miller, BSAVA Manual of Small Animal Fracture Repair and Management, Wiley, 6 mar 1998 – 332
[2] W.O. Brinker. The use of intramedullary pins in small animal fractures. North Am Vet 29:292, 1948
[3] E.A. Ehmer. Bone pinning in fractures of small animals. J Am Vet Med Assoc 110:14, 1947
[4] R.L. Rudy. Principles of intramedullary pinning. Vet Clin North Am 5:209. 1975

OTOIMPLANT - A NEW MIDDLE EAR PROSTHESIS AS AN ALTERNATIVE MEDICAL DEVICE IN OPERATIVE TREATMENT OF HEARING

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[ENGINEERING OF BIOMATERIALS 143 (2017) 47]

Introduction

The most common origins leading to the destruction of the continuity of ossicles chain connection are chronic inflammations and mechanical injuries [1]. Damage of ossicles continuity cause consequences that are manifested by conductive hearing loss. Partially or completely destroyed conductive apparatus of the middle ear requires surgical treatment using tympanoplastic techniques of chain reconstruction using grafts or adjustment and implantation of alloplastic implants [2]. New construction and material possibilities allow design and production of prostheses not only in various sizes and shapes, but also differentiated in terms of alloplastic materials, such as metals, ceramics, plastics or composites. Auditory ossicle prostheses are used to transmit sound or a sound signal from the tympanic membrane to the inner ear when the ossicles of the human middle ear are entirely or partially absent or damaged [3].

The use of new middle ear implants with bactericidal and bioactive properties may not only restore continuous bone structures and restore lost functions, but also may reduce recovery periods and risk associated with complications in the course of infection and bacterial infections.

Materials and Methods

Otoimplant - is an alternative to existing solutions offered on the market. It is made of a composite on a matrix of biostable medical polymer and silver nanoparticles. antibacterial and lightweight may be treated as innovative aspects of developed prosthesis. Implants made of polymer and silver nanoparticle-modified composite after the in vitro phase have been implanted in the buttock muscle of Wistar rats. After 30, 90 and 180 days, histochemical and histoenzimatic tissue specimens were evaluated. The last step was a clinical trial involving implantation of a prosthesis into the middle ear space of a patient suffering from hearing loss due to partial destruction of the auditory ossicles.

Results and Discussion

The *in vitro* studies indicate high antimicrobial efficacy of Gram-positive and Gram-negative bacteria. The *in vivo* studies have confirmed the biocompatibility of implants in the tissue environment. After 90 days of implantation, there is a decreasing granulation area showing the inflammatory process, and emerging regenerative muscle fibers. On the 6th of April 2017 the first pioneering operation of otoimplant implantation was performed during clinical trials at the University Hospital in Cracow. This operation has confirmed the effectiveness of the implant.

Conclusions

The surgical procedure of inserting the otoimplant into the middle ear spaces and the postoperative period run without complications. Patient reported subjective improvement in hearing.

Prosthesis designed for the reconstruction fitted perfectly and allowed precise implantation.

Proposed implant might be successfully used in present surgery. The first operation confirmed the effectiveness of the implant. Positive results of committed research will allow wide group of patients to get access to modern medical product. Cheaper, more accessible implants with bactericidal properties will support antibiotic treatment. Developed prosthesis will act like a barrier protecting from reoccurring illness and inflammation what will shorten necessary hospitalization and accelerate convalescence.

Acknowledgments

This work was funded by the National Research and Development Center as part of the project LIDER/ 154/L-6/14/NCBR/2015.

References

[1] T. Zahnert, Reconstructive methods in hearing disorders - surgical methods, GMS Curr Top Otorhinolaryngol Head Neck Surg. 2005; 4: Doc02.
[2] C. Milewski Composite graft tympanoplasty in the treatment of ears with advanced middle ear pathology. Laryngoscope. 1993;103:1352–1356.
[3] U. Steinhardt, H. Kurz, Auditory ossicle prosthesis with variable coupling surfaces**,** US8128694 B2.

CHITOSAN/SILVER LAYER DEPOSITED ON NiTi SHAPE MEMORY ALLOY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 48]

Introduction

The unique shape memory properties of the NiTi alloys make them one of the frequently used materials for medical application [1]. However, the remaining problem is their corrosion resistance due to the release of nickel, when they are applied in the biological environment. Attempts to improve corrosion resistance were primarily focused on the modification of alloy surfaces by the creation of protective layers/covers from: titanium oxides, titanium nitrides, hydroxyapatite, polylactide etc.
In the presented work, multifunctional approach to improvement of NiTi properties was done. The surface was cover with chitosan (CH) and silver composite. The silver is known from its antibacterial properties whereas chitosan can protect NiTi surface and follow deformation coming from the shape memory effect [3-4].

Materials and Methods

Commercial NiTi shape memory alloy was used as a substrate for CH/Ag layer production. On polished surface of the NiTi alloy, CH (Sigma Aldrich) simultaneously with Ag (AEE) was electrophoretically co-deposited using deposition voltage: 25, 30, 35 or 40V and deposition time from 60s to 120s.

Results and Discussion

Observations carried out with use of electron scanning microscope confirmed, that in all cases of deposition parameters, the surface of the alloy was covered with a thin layer composed of CH matrix with Ag as a composite component. Example of SEM images is shown in FIG. 1 and 2. In general, Ag particles were randomly distributed in the CH cover. Several of them formed agglomerates that protruded above the surface of CH. The diameter of the agglomerates increased with increasing of deposition voltage. Moreover, increasing deposition time and voltage resulted in the appearance of some discontinuities in the CH shell.

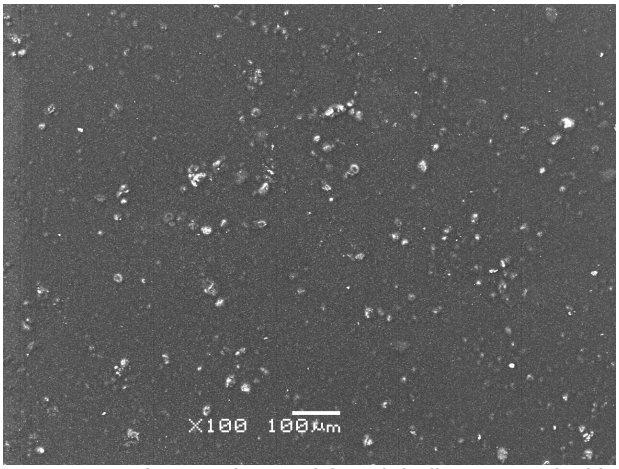

FIG. 1. SEM image observed for NiTi alloy covered with CH/Ag deposited at 25V/120s.

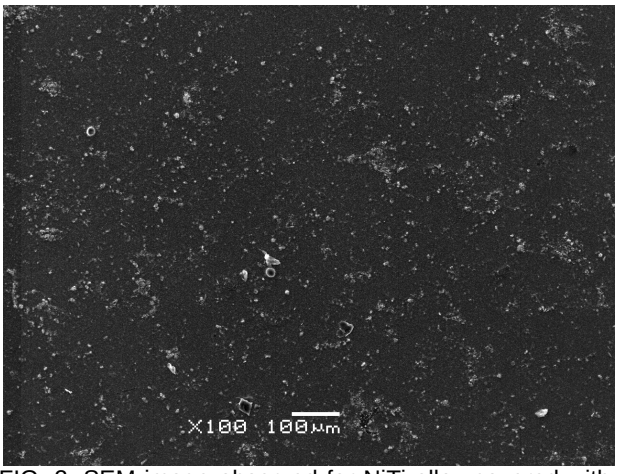

FIG. 2. SEM image observed for NiTi alloy covered with CH/Ag deposited at 40V/60s.

Structure of the cover was examined with use of X-ray grazing incident beam diffraction technique. Example of the measured diffraction patterns was shown in FIG. 3. Diffraction patterns measured at angle of 5 degrees revealed presence of lines belonging to Ag, mainly. The relatively high X-ray penetration depth revealed the presence of diffraction lines coming from the NiTi alloy substrate. Also, a widened maximum in the angular range of 15 to 25 degrees indicates the presence of CH in the amorphous form. Reducing the penetration depth of the x-ray beam (angle of incident beam was 0.3 deg) allowed to obtain the diffraction pattern only from the layer. Phase identification confirmed the presence of CH and Ag in the coating.

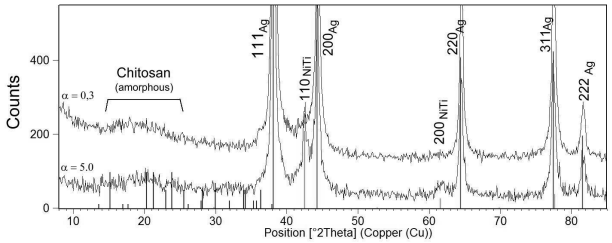

FIG. 3. GIXD patterns measured for NiTi alloy covered with CH/Ag at 40V/60s.

Conclusions

The increase in deposition voltage caused the formation of ever larger agglomerates consisted of Ag particles. Elongation of the time of electrophoretic deposition resulted in a discontinuity of the CH coating. This effect was especially visible on the edges of the sample.

References

[1] T. Yoneyama, S. Miyazaki, Shape memory alloys for biomedical applications, Woodhead Publishing Limited, Cambridge (2009).
[2] J. Lelątko, T. Goryczka, Modyfikacja powierzchni stopów NiTi wykazujących pamięć kształtu, Oficyna Wydawnicza WW, Katowice (2013).
[3] L. Ge, Q. Li, M. Wang, J. Ouyang, X. Li, M.M. Xing, Int. J. Nanomedicine 9 (2014) 2399–2407.
[4] J.A. Jennings, J.D. Bumgardner, Chitosan Based Biomaterials Volume 2: Tissue Engineering and Therapeutics, Woodhead Publishing, (2016).

CARBON ALLOTROPES FOR MUSCLE REGENERATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 49]

Introduction

Stem cells, developing in the body, are surrounded by a specific micro-environment of the adjacent cells and components of the extracellular matrix, constructed from proteins and proteoglycans [1]. This environment of stem cells is called stem cell niche, a key factor for their further proliferation and differentiation, including *in vitro* culturing. The standard methods of cell culturing include only an effect of the two-dimensional substrate surface. The lack of the interaction between a niche and a stem cell in the 3D contact represents a significant limitation and difficulty in the *in vitro* tissue culture and especially in the *in vitro* culturing of muscles. The creation of the 3D structure that allows for culturing of muscle cells from their progenitor cells could represent a breakthrough in developing methods for *in vitro* cultivation of muscles' implants. The 3D construction composes an architectural system similar to the natural, including mechanical functions that mimic the basal lamina and extracellular tendon matrix function.
Carbon allotropes, as a 3D scaffold of the niche, can create the perfect environment for cell growth, particularly, because it is a highly biocompatible material [2]. Above all, they can be a relatively simply functionalised, hence, different organic molecules can be attached to them, in particular, proteins (amino acids), imitating natural niche cells [3].

Materials and Methods

Carbon scaffolds were prepared by layer placement and desiccation of the colloids of nanoparticles of diamond (ND), fullerenes (F60), nanotubes (NT), nanotubes OH (NTOH), nanotubes COOH (NTCOOH), graphene oxide (GO) on the bottom of a culture flask. Mesenchymal stem cells were collected from the hind limb bud of chicken embryos. On day 7 embryos were sterile removed from the eggs and a bud of the hind leg was collected, using a microscope, and placed gently in a solution of trypsin, then put aside in the refrigerator for a 24 h. In the next step, the solution was neutralized by the addition of a standard culture medium (DMEM - Dulbecco's Modified Eagle's), gently stirred and placed in flasks (BD) or dishes and culture, according to particular experiments.
Culture medium was changed every 3rd day. Cells were maintained in DMEM culture medium containing 10% foetal bovine serum (Life Technologies, Houston, TX, USA) and 1% penicillin and streptomycin (Life Technologies) at 37°C in a humidified atmosphere of 5% CO_2/95% air in a DH AutoFlow CO_2 air-jacketed incubator (NuAire, Plymouth, MN, USA). In the experiments, high or low glucose, L-glutamine, DMEM were used. At day 5 the morphology of mesenchymal and muscle cells was visualized, using SEM and light microscopes. Expression of the mRNA of chosen proteins was measured.

Results and Discussion

Morphology of mesenchymal muscle cells and their interaction with carbon scaffolds are observe using SEM and light microscopes. The interaction of nanoscaffolds with cells differed and the morphology; number and state of the development of cells were influence by carbon allotropes. The most neutral for stem cells were nano-diamond based scaffold. The scaffold, prepared from fullerenes, was the most colonised by cells, moreover, it stimulated cell proliferation. The scaffold constructed from carbon nanotubes, functionalised with COOH, was also well settled by cells, better than scaffold with nanotubes and nanotubes OH. GO scaffold stimulated differentiation of muscle stem cells and creation of the muscle tissue (FIG. 1). mRNA expression of muscle cells colonised in GO scaffold clearly showed increased expression of MyoD-marker of differentiated muscle cells.

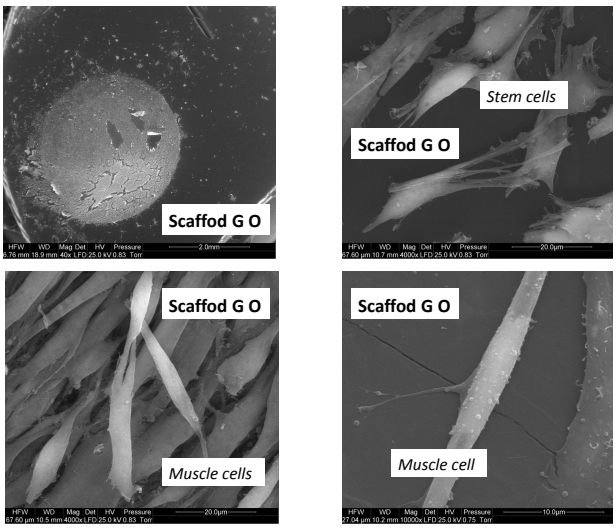

FIG. 1. Morphology of the interaction between cells and scaffolds.

Conclusions

Nano-scaffolds, depending of the carbon allotropes, influenced behaviour and morphology of muscle stem cells, moreover, their functionalization (nanotubes) changed bio-function of scaffolds and stem cells number and morphology. GO scaffold stimulated mesenchymal stem cells differentiation and beginning of the formation of muscle tissue.

Acknowledgments

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References

[1] Seeger T., Hart M., Paatrroyo M., Rolauffs B., Aicher K., Klein G. 2015. PLoS One, 9. e0137419.
[2] Kurantowicz N., Strojny B., Sawosz E., Kutwin M., et al., 2015 Nanoscale Res. Lett. 10, 398.
[3] Sawosz E., Jaworski S., Kutwin M., Vadalasetty KP., Grodzik M. et al., 2015. Int. J. Mol. Sci. 16, 25214-33.

CELLULOSE AS A MATRIX FOR SYNTHESIS OF THE LIBRARY OF MOLECULAR RECEPTORS USEFUL FOR SCREENING OF ANTIHISTAMINE COMPOUNDS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 50]

Introduction

Cellulose is a biopolymer composed of β D-gluco-pyranose residues bonded with 1,4-glycosidic bonds. Its characteristic feature is the equatorial arrangement of secondary hydroxyl groups C2 and C3 and also the primary hydroxyl group C6. The result of this arrangement is the regular structure of cellulose resulting from the high content of crystalline phase [1]. In addition, the reactivity of particular hydroxyl groups is clearly defined. It is assumed that primary hydroxyl groups are about 25 times more reactive than secondary Presented facts make cellulose and its derivatives widely applicable [2]. It has also been shown that cellulose can be used as a membrane for bounding on its surface *N*-lipidated peptides which are able to mimic natural receptors and/or enzymes [3,4] by formation of binding cavities which are able to interact with different ligands recognizing their size, shape, charge distribution, chirality and polarity. Process of binding is reversible due to the nature of interactions between ligands and the binding pockets and it has been found that mechanism of binding is competitive and therefore the described process is mimicking the interactions involving natural receptors [5]. Herein we present an attempt to test whether a library of peptides immobilized on cellulose can mimic a histamine receptor and thus be used in studies with antihistamine active compounds. It is expected that it would be possible to select molecular receptors selectively interacting with agonists and antagonists.

Materials and Methods

Whatman-7 filter paper was used as matrix in the study. Cellulose was modified with 2,4-dichloro-6-methoxy-1,3,5-triazine and *m*-phenylenediamine according to standard protocol [5]. Syntheses of *N*-lipidated immobilized peptides were made by automated SPOT methods using as a coupling reagent DMT/NMM/TosO$^-$. In all cases, two identical libraries were synthesized on each cellulose sheet. After splitting for two parts, one of them was treated with active substance and then with reporter dye (Brilliant Black), the second one was used for bounding the reporter dye only. For docking studies were used Histamine, Diphenhydramine, Doxylamine, Cimetidine, Ranitidine. All cellulose sheets after experiments were dried, scanned, and processed using Image-Quant program. Ability of molecular receptors to interact with colorless active compounds was calculated as difference in intensity of coloration by reporter dye and intensity of coloration after treatment with colorless ligand and subsequently with reporter dye. In this way, for each spot was determined average value of "gray" coloration calculated corresponding to interaction between binding pocket of molecular receptor and antihistamine ligand.

Results and Discussion

In order to study a representative number of peptide structures involved in the formation of molecular receptors were used modified SPOT methodology. *N*-Lipidated peptides were immobilized on cellulose *via* aromatic linker containing fragments of *m*-phenylenediamine and 1,3,5-triazine connected with the cellulose surface in highly selective reaction with primary hydroxymethyl groups (FIG. 1).

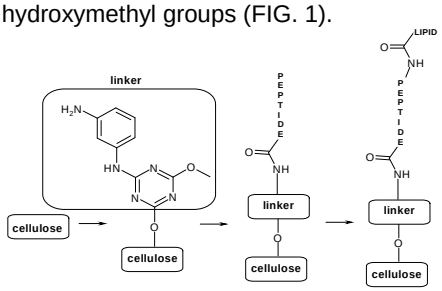

FIG. 1. Methods of synthesis of *N*-lipidated peptides immobilized on the cellulose.

In this studies were prepared the randomised library of *N*-heptanoylated dipeptides. As a *C*-terminal amino acids were used: alanine, proline and phenylalanine, as *N*-terminal residues were applied all natural amino acids. Finally, it was synthesized 60-elemets library of molecular receptors. As ligands for docking processes were applied compounds with a documented H1-H4 agonistic and antagonistic activity and Histamine as a natural ligand. As agonists were used Diphenhydramine and Doxylamine, as antagonists: Cimetidine and Ranitidine. The acquired results shown that binding pockets created by *N*-heptanoylated peptides are able to selective binding of tested anitihistamine compounds (FIG. 2).

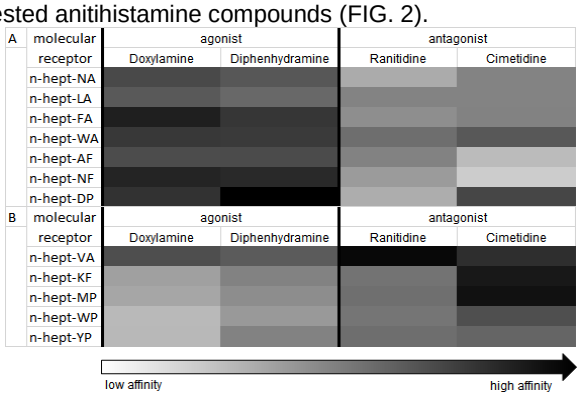

FIG. 2. Map of interaction between molecular receptors and tested compounds, A panel – strong bounding of agonists; B panel – strong bounding of antagonists.

Conclusions

These studies revealed that library of molecular receptors is capable in recognition and differentiation of agonistic/antagonistic profile of antihistamine active compounds. Even not understanding of complex relations between the structure of the molecular receptor and structure of the pharmacologically active substance, this should allow the construction of a new research tool useful as a platform for screening of new antihistamine compounds.

Acknowledgments

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References

[1] D. Klemm, B. Heublein, *et al.,* Angew. Chem. Int. Ed. 44 (2005) 3358-3393.
[2] J. George, S.N. Sabapathi, Nanotechnol Sci Appl. 8 (2015) 45-54.
[3] J. Fraczyk, B. Kolesinska, Z.J. Kaminski, Comb. Chem. & HTS 16 (2013) 562-571.
[4] A. Bak, M. Daszykowski, *et al.,* Comb. Chem. & HTS 17 (2014) 141-156.
[5] J. Fraczyk, Z.J. Kaminski, J. Comb. Chem. 10 (2008) 934-940.

A NEW STRONTIUM AND ZINC DOPED BIOGLASSES FOR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 143 (2017) 51]

Introduction

Bioglasses are materials known for the ability to create a strong bond with tissues, especially bone tissue. Numerous studies revealed beneficial effects of strontium on bone tissue such as increasing osteoblasts proliferation or stimulating bone remodeling [1], whereas zinc is reported to have antibacterial performance [2]. The aim of the study was to describe influence of the glass modifiers (strontium and zinc) on the glass structure and *in vitro* bioactivity. What is more, their influence on the growth and differentiation of bone and cartilage tissue cells have been investigated.

Materials and Methods

Glasses from the SiO_2-CaO-P_2O_5-SrO and the SiO_2-CaO-P_2O_5-ZnO systems have been obtained by the sol-gel route. They were differing in CaO/SiO_2 ratios and the concentration of modifier oxides (SrO and ZnO), varying between 0-5 % molar percents. Materials were subjected to the structural analyses (XRD diffraction, FTIR spectroscopy, NMR spectroscopy). Moreover, *in vitro* bioactivity tests in simulated body fluid solution were performed. For the biological test purposes glass powders were incorporated into PCL polymer matrix with the 50% glass particles weight fraction The series of *in vitro* biological tests was made in order to evaluate the influence of the additives type and concentration on the bone (NHOst Lonza) and cartilage (NHAC, Lonza) tissue cells phenotype and the behavior. Osteoblasts culture was analyzed for cytotoxicity, level of ALP, and extracellular matrix mineralization level. Moreover, cells morphologies were evaluated by acridine orange fluorescent staining. Chondrocytes were analyzed for collagen type II level and aggrecan expression.

Results and Discussion

Structural analyses of obtained glasses changes occurring in the materials structure along with the introducing of SrO or ZnO in place of CaO. Sr incorporation effected in the increase in the amount of the non-bridging Si-O- bonds, what was probably an effect of the differences in size between Ca^{2+} and Sr^{2+} cations. Our study has shown that the effect of strontium oxide on the structure and properties of gel-derived biomaterials largely depended not only on SrO concentration but also on the chemical composition of starting materials.

Bioactivity *in vitro* tests indicated that all of obtained materials were bioactive, but the dynamics of the process and bioactive layer morphology depended on the bioglass composition.

Biological tests indicated that incorporation of Sr and Zn to glasses significantly affects cell behavior and phenotype. Strontium containing glasses favored osteoblast cells differentiation and increased ALP activity, whereas both Zn or Sr-containing glasses. Results of the mineralization assay *in vitro* performed on the osteoblast cells after 21 days of culture indicated that the highest level of ECM mineralization exhibited cells cultured in the material with Sr-containing glass particles. Moreover, modifying bioglass with strontium has significantly improved ECM mineralization in comparison with other materials, especially with TCPS and bare PCL film. Studies of the collagen type II level produced by the chondrocytes cultured on the model bioglass/PCL composite films indicated that after 7 day of culture the total collagen production was similar for all tested materials. After 14 days the level of produced collagen type II in A2/PCL and PCL materials was lower than for the TCPS, whereas the highest level of production was detected for the material containing glass doped with 5% mol of zinc what indicated that Zn-doped bioglasses stimulated chondrocytes to collagen type II production better than other tested materials.

Conclusions

Our study has confirmed that addition of strontium and zinc to glasses can improve their biological response in vitro in contact with various cell lines.

Acknowledgments

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

References

[1] L. Wei, J. Ke, *et.al*. I., Osteoporos. Int. 25 (2014) 2089–2096. doi:10.1007/s00198-014-2735-0.
[2] A. Hoppe, N.S. Güldal, A.R. Boccaccini, Biomaterials. 32 (2011) 2757–2774. doi:10.1016/j.biomaterials.2011.01.004.

EFFECT OF SILVER NANOPARTICLES ON CHICKEN HEALTH AFTER INFECTION WITH *CAMPYLOBACTER JEJUNI*

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[ENGINEERING OF BIOMATERIALS 143 (2017) 52]

Introduction

Silver nanoparticles (AgNP) have gained much attention in recent years due to their biomedical applications, especially as antimicrobial agents. AgNP may be used in poultry production as an alternative to the use of antibiotic growth promoter. However, little is known about the impact of oral administration of AgNP on the gut microbiota and the immune system. The aim of the present study was to investigate the effects of AgNP on growth, hematological and immunological profile as well as intestinal microbial composition in broilers challenged with *Campylobacter jejuni* (*C. jejuni*).

Materials and Methods

Ninety day-old broiler chickens were randomly assigned to two groups: control and provided with AgNP in the drinking water (50 ppm). At day 11, all birds were orally challenged with an overnight *C. jejuni* culture. Feed consumption, water intake, body and organ weights were registered to evaluate the influence of AgNP on chicken performance. The *in vivo* antibacterial activity of AgNP was assessed by using plate count method by measuring packed cell volume (PCV) percent in chicken blood samples using the micro-hematocrit reader. Humoral immune status was determined by measuring plasma immunoglobulin concentrations. The effect of AgNP on the inflammatory response was measured in liver tissue samples by mRNA expression of TNF-α and NF-kB using qPCR analysis.

Results and Discussion

AgNP did not affect the intestinal microbial profile of birds. These results are consistent with *in vivo* experiments with the microbial profile of young quails receiving hydrocolloids of AgNP administered with 5-25 ppm [1]. The obtained results may suggest that AgNP were gastro-sensitive, the stability and dispersion of AgNP in gastric acid is a critical factor for antibacterial activity.

The body weight gain and the relative weights of bursa and spleen were reduced when supplemented with AgNP. The results are consistent with decreased body and organ weights in chickens treated with 25 ppm of AgNP [2]. The PCV results indicated that the provision of AgNP did not influence the percentage of red blood cells. On the other hand, it was reported that the oral administration of AgNP induced some changes in the red blood compartment, such as increased red blood cell count and coagulation parameters [3].

The plasma concentrations of IgG and IgM were lower in birds receiving AgNP compared to the non-supplemented control group (FIG. 1) AgNP might impair intestinal actively transported sugars, amino acids, trace elements, and vitamins, and deficiencies of these nutrients may decrease antibody formation. Similar observations showed decreased plasma IgG levels in chickens treated with 10 and 20 ppm AgNP but not infected with *C. jejuni* [4].

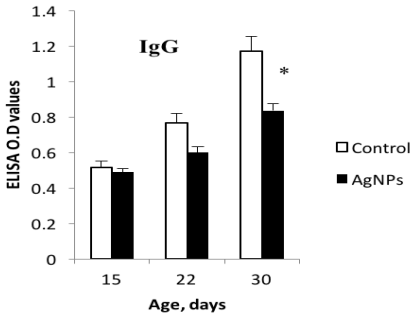

FIG. 1. Concentration of immunoglobulins (IgG) in chickens infected with *C. jejuni* * Indicates significant difference between control and AgNP ($p < 0.05$).

The expression of *TNF-α* and *NF-kB* at mRNA level was significantly higher in birds receiving AgNP. An increase in mRNA expression of inflammatory mediators and low IgG and IgM levels could be due to the nanoparticle uptake triggering cellular effects, leading to inflammatory responses. However, the conflicting results might indicate that AgNP have multiple cellular targets that vary among different cell type. These results are attributed to several confounding factors such as pH [5], continuous oral administration of AgNP, or even the availability of free radicals to induce oxidative stress and damage cells [3,6]. We propose that nanoparticles time of exposure, route of administration, particles size, aggregate formation, and altered bio-distribution in the form of rapid clearance owing to non-specific pathogen clearance from the systemic circulation could serve as aided factors. One possible cause for the AgNP dependent initiation of inflammation could be the fact that they enhance the production of reactive oxygen species. These oxygen-derived free radicals may lead to mitochondrial dysfunction, increased gene expression of inflammatory cytokines (TNF-α) and activation of specific transcription factors (NF-kB).

Conclusions

The application of AgNP via the drinking water in the concentration of 50 ppm reduced chicken growth, impaired immune functions and had no antibacterial effect on different intestinal bacterial groups, which may limit the applicability of AgNP against *C. jejuni* in broiler chickens.

Acknowledgments

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References

[1] E. Sawosz, M. Grodzik *et al*., Arch. Geflügelk. 73 (3) (2009) S208–213.
[2] F. Ahmadi, A.H. Kurdestany, Global Veterinaria 5(6) (2010) 366-370.
[3] Y.S. Kim, JS. Kim *et al.,* Inhal. Toxicol. 20(6) (2008) 575-583.
[4] L. Pineda, A. Chwalibog *et al.,* Arch. Anim. Nutr. 66(5) (2012) 416-429.
[5] P.L. Nadworny, J.F. Wang *et al.,* J. Inflamm. 7(13) (2010) 1-20.
[6] E.J. Park, E. Bae *et al.,* Environ. Toxicol. Pharmacol. 30 (2) (2010) 162-168.

COMPARATIVE ANALYSIS OF POROUS POLYMERIC MEMBRANES AS DRUG CARRIERS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 53]

Introduction

Porous polymer membranes are potential multi-level carriers of bioactive substances or drugs. Their release depends on the type of polymer matrix, and the longer the time of degradation of the matrix, the lower the release rate. Biodegradable polyhydroxyl acids, represented by polylactide (PLA) and aliphatic polyesters including polycaprolactone (PCL), have been used as drug shell for drug delivery. These polymers can be easily formed into granules, fibers or membranes. For example membrane materials can be obtained by the phase inversion method which allows to control the surface and volume porosity. It depends on the conditions of the coagulation bath and the concentration of the polymer.

Two polymers were used as the template: polylactide (PLA) and polycaprolactone (PCL), which were introduced into biofuroxime (Bf) (second generation cephalosporins). The presence of this compound has bactericidal activity against both Gram-positive and Gram-negative bacteria. The efficiency of the modification was confirmed by the SEM/EDS observation. Drug release was monitored by changing the analytical concentration of ions (ICP method) and parallel study in contact with Gram-positive bacteria was conducted.

Materials and Methods

Commercial polymers: PLDLA (Carbochem) and PCL (Sigma-Aldrich) were used in the experiment. Mixture of AC and THF (1:8) was used as the solvent. The precipitating reagent was DMSO. All reagents were purchased from Avator (Poland). The dissolved polymers were doped with 5% wt of biofuroxime (Polfa). The membranes were air dried and then vacuum treated for 48h. Microstructure of membranes was observed by scanning electron microscope (Nova NanoSEM). Other features of membrane were tested during permeability test, drug release (ICP), and durability *in vitro* (PBS/3msc/37°C). Multi-level carrier membranes were tested using an agar-based method. Pure (unmodified) polymeric membranes were the reference in all studies.

Results and Discussion

Addition of the modifier affected microstructure, and the size of pores was reduced: for the PCL-based polymer membrane the diameter of pores decreased from about 60 µm (for PCL) to 22 µm (for PCL/Bf). The same effect was observed in the PLDLA membranes, and the average size of pores decreased from 20 µm (for PLDLA) to 12 µm (for PLDLA/Bf). Release of biofuroxime from the PLDLA membrane was faster than that of the PCL as confirmed by increase in concentration of sulfate ion (analytical group of drug). Drug release process during incubation of PCL/Bf membrane started after 14 days, which resulted from lower degradation rate of the porous PCL membrane. All membranes were characterized by altered morphology (irregular pores with a rough surface), and slight changes in weight and dimensions after degradation.

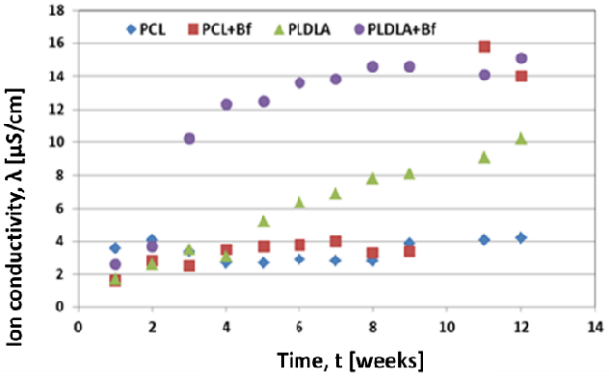

FIG. 1. Stability of polymer membrane materials: PLDLA, PLDLA/Bf and PCL, PCL/Bf.

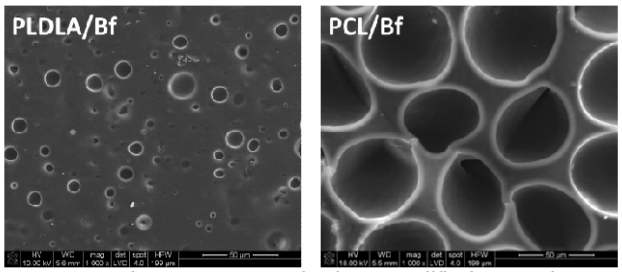

FIG. 2. Microstructure of drug-modified membrane materials (biofuroxsim)

Conclusions

Phase inversion is a method of obtaining polymer membranes, which can be modified with bioactive compounds and drugs. This preliminary study has shown that a more stable PCL matrix releases drug later than the faster degrading PLDL matrix. Both PLDLA and PCL porous membranes were stable for not longer than 3 months.

Acknowledgments

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References

[1] A.Rapacz-Kmita, E.Stodolak-Zych, B.Szaraniec, M.Gajek, P.Dudek, Mater. Lett. 146 (2015) 73-76
[2] A.Rapacz-Kmita, M.M.Bućko, E.Stodolak-Zych et al., Mater. Sci. Eng. C 70 (2017) 471-478
[3] A.Rapacz-Kmita, E.Stodolak-Zych, M.Dudek et al., J. Therm. Anal. Calorim 127 (2017) 871-880
[4] A.Rapacz-Kmita, M.Gajek, M.Dudek, E.Stodolak-Zych et al., J. Therm. Anal. Calorim 127 (2017) 389-398

DEVELOPMENT AND OPTIMIZATION OF MYOCARDIAL TISSUE CULTURE IN OVO

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[ENGINEERING OF BIOMATERIALS 143 (2017) 54]

Introduction

Characterization of cells in terms of their usefulness in regenerative medicine is based on research on their potential for differentiation and proliferation in vivo. The chorioallantoic membrane (CAM) of chicken embryo is an optimal environment for growing xenografts taken from other individuals or even species. In research conducted so far human stem cells were implanted directly into different parts of the embryo (eg. neural tube) as well as systemically through injections to veins and arteries of CAM [1]. It was also demonstrated their integration into the organism in the late stages of embryo development [2]. CAM as a biological model with natural immunodeficiency has already become a useful tool for breeding and researching many cancers, with no restrictions on the species [3-5]. The CAM model was also used for implantation of tissue fragments. In this system, however, the survival of transplanted tissues is dependent on rapid neovascularization, the failure of which results in death of the transplant. Transplantation of adult tissues did not lead to revascularization of their fragments probably due to lack of ability to stimulate host angiogenesis as it is for tumour tissue [6]. The positive effect augurs a use in place of the adult tissue, cells or tissues of a progenitor character. Both adult and embryonic stem cells provide an excellent tool for cell therapy. They owe the ability to differentiate into somatic cell lines [7]. The control of this differentiation is mainly achieved by direct stimulation of their surface receptors with appropriate transcription factors [8,9]. Previous studies on the chicken embryo CAM model and broiler model in vivo carried out with nanoparticles of colloidal silver showed their strong proangiogenic effects due to increased expression of angiogenic factors (VEGFA, FGF2) [10]. Their influence on the morphology of chick embryos was also reported [11]. The advantage in the case of xenografts implantation is also the proven antiseptic effect of silver.

Excellent blood supply, oxygen access and optimal physico-chemical conditions allow for effective cell culture, which can be differentiated and transformed into tissue. Progenitor cells implanted on the chorioallantoic membrane of the chicken embryo at different stages of development allow to determine which degree of differentiation of implants is optimal for these applications. The aim of the experiment is to determine the influence of in ovo culture conditions, on the size, structure and morphology of the resulting fragments of tissue. The use of nanocolloid of silver as a proangiogenic factor in relation to the cardiac progenitor cells implanted on the CAM could have a positive effect on vascularisation of obtained tissue fragments, which in turn would improve their growth and survival in ovo.

Materials and Methods

The model organism was CAM of a chick embryo and chicken embryos bred under standard conditions until the 18th day of embryonic development. Cardiac progenitor cells were collected at 6th and 18th ED. On each of these days eggs were opened and the embryos were sacrificed. Hearts were immediately isolated, tripsinised, homogenised and mixed with the culture medium to neutralise the enzyme. They were cultured in vitro to check their reaction to the Ag nanoparticles in medium or implanted as a primary cultures on the chorioallantoic membrane of chicken embryo at 8th ED with and without the addition of Ag nanoparticles as pro-angiogenic factor, which were administered in the vicinity of the implanted cells at the time of implantation. The size and morphology of the grown tissue fragments were assessed by macroscopic images from the binocular immediately after their isolation.

Results and Discussion

Preliminary experiment with the nanocolloidal Ag has shown that in a limited range of concentrations (2 and 10 ppm) of the silver nanoparticles, in the short term they stimulate cells' metabolism. In the lower concentration they cause formation of three-dimensional structures and prolong the life of cardiac progenitor cells in culture. As transcriptionally active they can potentially initiate self-renewal of cells or stimulate differentiation of pluri/ multipotent cells. Morphology of primary cells in culture differs depending on the cells' age. In turn, studies carried out on cardiac progenitor cells from chicken embryos (ED 6 and 18) after implantation on CAM of another embryo confirmed the formation of small tissue fragments within the chorioallantoic membrane. Cells collected on day 6th of embryo development after implantation in ovo migrate further from the injection site than 18 ED cells. Cells derived from 6th ED remain alive until the end of the culture in ovo.

Conclusions

Heart-derived progenitor cells taken from the embryo of chicken at 6th and 18th embryonic development days after implantation on chorioallantoic membrane form in ovo vascularised spatial structures. The 15 µg Ag nanoparticles supplementation at cells' implantation time results in more orderly cell proliferation towards heart-like structures.

Acknowledgments

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References

[1] Boulland et al. J. Vis. Exp.2010 (41), e2071, doi:10.3791/2071
[2] Schomann et al. Stem Cells International 2013, vol. 2013, Article ID 960958, 9 pages
[3] Balke et al. BMC Research Notes 2010, 3:58
[4] Urbańska et al. Nanoscale Res. Lett. 2015, 10:98.
[5] Sys et al. J. Vis. Exp. 2013 (77), e50522, doi:10.3791/50522
[6] Ribatti D.ed. 2010, Use of Chorioallantoic Membrane in the Study of Vascularization of Grafted Tissues, in: The Chick Embryo Chorioallantoic Membrane in the Study of Angiogenesis and Metastasis. Springer Dordrecht Heidelberg London New York
[7] The Cell Biology of Stem Cells, ed. Meshorer E. and Plath K. Landes Bioscience / Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology.
[8] Dalziel et al. PNAS, 2013, 110(44):17608–17609
[9] Deasy et al. Molecular Therapy 2009. v 17 no. 10.
[10] Hotowy et al. Nanoscale Res. Lett. 2012, 7, 418
[11] Sawosz et al. Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 2012, 51.

THE INFLUENCE OF SATURATED FATTY ACIDS ON HUMAN LUNG EPITHELIAL CELLS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 55]

Introduction

Fatty acids (FAs) can be utilized in manufacturing of novel inhalable drug delivery systems for lung cancer treatment, i.e. solid lipid nano- or microparticles [1]. FAs are naturally occurring in saturated or unsaturated forms with different carbon chain lengths. It is known that unsaturated FAs (i.e. arachidonic acid) decrease cell membrane stiffness of lung epithelial cells, leading to increased drug uptake and bioavailability [2]. Unsaturated FAs are in liquid state at room temperature so they cannot be used as nano- or microparticle matrix. Saturated FAs are promising materials for fabrication of drug delivery carriers, however there is limited information on their effect on lung epithelial cells.
The aim of this study was to evaluate the influence of various saturated FAs on viability and mechanical properties of malignant and non-malignant human lung epithelial cells.

Materials and Methods

Human lung epithelial cells (malignant – A549. ATCC® CCL-185™ and non-malignant – BEAS-2B, ATCC® CRL 9609™) were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 1% glutamine (only for BEAS-2B). Cells were seeded in 96-well plates or Petri dishes and cultured overnight prior to addition of FAs. FAs (C10:0 – C18:0) were dissolved in 99.8% ethanol and added to cell culture medium to obtain final concentrations of FAs equal to 25, 50, 75 and 100 µM (final concentration of ethanol <1%). The ratio of cell number to the amount of FAs added was constant in all the experiments. FAs uptake was determined using optical tomography (Nanolive 3D Cell Explorer). Viability of cells after 24 h incubation with FAs was evaluated using resazurin reduction assay (AlamarBlue, Sigma-Aldrich) and live/dead fluorescent staining. Cell proliferation was assessed using IncuCyte® ZOOM System (Essen BioScience) that records phase contrast images of cells every 2 h. Cell membrane stiffness was determined by atomic force microscopy in contact mode with 10 nN indentation force (MFP-3D-Bio, Assylum Research).

Results and Discussion

FAs were easily uptaken by both malignant and non-malignant cells, however the amount of fatty acids stored in lipid droplets within the cells was higher in malignant cells (FIG. 1). Metabolic activity assays showed that several saturated FAs (i.e. myristic and palmitic acids) at the lowest concentration of 25 µM decrease viability of malignant epithelial cells (<60% compared to control), but they are non-toxic for non-malignant epithelial cells. FAs such as capric and lauric acids did not affect both malignant and non-malignant cells growth even at the highest concentrations (up to 100 µM). These findings were confirmed by live/dead staining and determination of cell proliferation over 4 days of incubation with FAs.

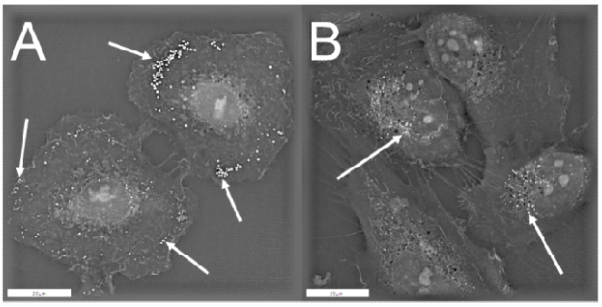

FIG. 1. Optical tomography images of malignant (A) and non-malignant (B) cells incubated with myristic acid for 24 h. Arrows indicate lipid droplets inside cells. Scale bar: 20 µm.

Cell membrane stiffness (Young's modulus) of cells incubated with FAs at 25 µM were determined based on the analyses of force-distance curves recorded using AFM. In the case of non-malignant cells, mechanical properties of cells incubated with various FAs were not significantly different from cells cultured in control conditions. However, when malignant cells were incubated with lauric, myristic and palmitic acids, the median Young's modulus of their cell membrane was almost twice lower than in control samples (FIG. 2). It will be further evaluated if changes in cell membrane properties result in increased membrane permeability.

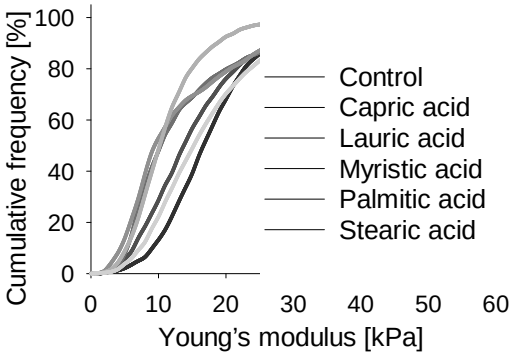

FIG. 2. Young's modulus of malignant cells incubated with saturated FAs.

Conclusions

The influence of saturated FAs on human epithelial cells was evaluated in this study. As natural substances, FAs are easily incorporated inside cells and stored in lipid droplets. The FAs uptake is more efficient in malignant cells than in non-malignant cells. Myristic and palmitic acids are toxic for malignant cells, even at low concentrations (25 µM), while being well tolerated by non-malignant cells. What is more, such FAs significantly decreased mechanical stiffness of cell membranes in malignant cells. This phenomenon may be beneficial in terms of novel lung cancer treatment, as the use of selected FAs for manufacturing of inhalable drug delivery systems can increase permeability of malignant cells, enhance drug uptake and result in more efficient treatment.

Acknowledgments

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References

[1] Jaspart, S., et al., Eur J Pharma Biopharma, 2007. 65(1):47-56.
[2] Haghi, M., et al., J Mater Chem B, 2015. 3(13):2583-2589.

PREPARATION AND CHARACTERIZATION OF POLYOXYMETHYLENE/ FUNCTIONALIZED HYDROXY-APATITE NANOCOMPOSITES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 56]

Introduction

Polyoxymethylene (POM) is one of an engineering thermoplastic polymers that is characterized by excellent mechanical properties, low friction coefficient and high chemical resistance to most solvents (1). POM is commonly used to replace metal and glass parts in automotive industry, industrial and medical equipment, consumer goods and parts of domestic appliances (2). It is usually manufactured by the copolymerization of trioxane with cyclic ethers and processed by extrusion and injection moulding methods (3). However, the biggest disadvantage of POM is its poor thermal stability. In elevated temperature it readily undergoes thermal decomposition. Furthermore, POM tends to follow an "unzipping" process with emission of formaldehyde molecules (4). Formaldehyde presence limits the range of application of POM in medicine in particular. To improved the thermal endurance of POM, a functionalized hydroxyapatite as a new kind of thermal stabilizer was applied.

Materials and Methods

POM copolymer (Ultraform®, BASF) was used as a composite matrix. Hydroxyapatite (HA, nGimat Co) in the shape of nanopowder was functionalized with poly(ethylene glycol) (PEG 2000, Sigma Aldrich). 1,6-hexamethylene diisocyanate (HDI) as a coupling agent, and dibutyltin dilaurate (DBTDL) as a catalyst were used (both form Sigma Aldrich). Anhydrous N,N-dimethylformamide (DMF, Avantor) was used as a solvent of the functionalization reaction. HA/HDI/PEG molar ratio was 1:2:1.

Grafting process of HA-g-PEG

HA/DMF (9 g/90 ml) was dispersed using sonication. Then, 9 µl of DBTDL catalyst was introduced to HA dispersion. Next, HDI/DMF (6 g/12 ml) solution was dropped to HA dispersion. The mixture was heated up to 80°C using magnetic stirrer and it was kept in this temperature for 1.5 h. After cooling down, PEG/DMF (36 g/36 ml) was dropped to the suspension and the system was heated up to 65°C and stirred in this temperature for 1.5 h. Finally, the powder was separated in centrifugal separator and washed three times with ethanol. After that, the HA-*g*-PEG powder was dried at 40°C for 24 h.

Processing of POM/HA-g-PEG composites

In the first stage, POM and HA-*g*-PEG powder were mechanically mixed (0, 0.5, 1.0, 2.5, 5.0 and 10.0% w/w of HA-*g*-PEG) (calculated in relation to pure HA). Then, composites were compounded in a twin-screw extruder (50 rpm, 210°C) and shaped by injection moulding method (210°C, 10 Bar). The composites were characterized using DSC and TG methods. FTIR and XRD analyses were also performed. The mechanical strength was measured using tensile test. SEM was applied to investigate the surface morphology of fractured specimens. The formaldehyde release during incubation was assessed using Schiff's reagent.

Results and Discussion

FTIR spectroscopy proved the urethane bond formation presence between –OH from HA and –NCO groups. SEM observations (FIG. 1) showed high cohesion between POM matrix (2) and the HA-*g*-PEG additive (1). DSC analysis confirmed that HA-*g*-PEG additive does not affect the crystallinity of POM relevantly, but supercooling of POM composites was lower, even by 4°C (for 10% HA-*g*-PEG content), compared with pristine POM. Most importantly, as can be seen in FIG. 2, the thermal stability of modified POM was increased up to 16.5°C (for 5% HA-g-PEG). Using the Schiff's test, the small amount of formaldehyde, comparable with distilled water, was detected in all samples. Mechanical tests exhibited some decrease in tensile strength from 70 MPa to 60 MPa for 10% of HA-*g*-PEG contents.

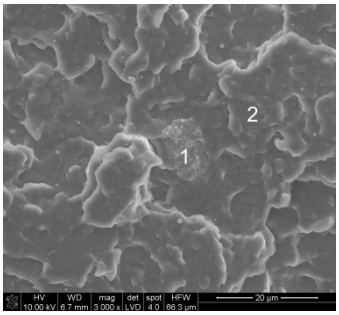

FIG. 1. SEM microphotographs for POM/ 1% HA-g-PEG nanocomposites

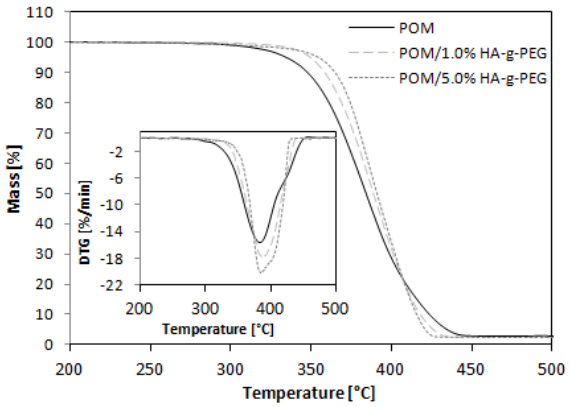

FIG. 2. TG/DTG curves of POM/HA-*g*-PEG composites (0%, 1%, 5%).

Conclusions

In this study, polyoxymethylene/functionalized HA composites with improved thermal stability were obtained. High mechanical properties and good stability of modified POM make this polymer material a great candidate as a material that can be used in many orthopedic applications.

Acknowledgments

The authors are grateful to the Polish Ministry of Science and Higher Education for financial support under the Grant No. 2016/21/B/ST8/00449.

References

[1] S. Lüftl, P.M. Visakh, Polyoxymethylene: State of Art, New Challenges and Opportunities, in: Polyoxymethylene Handbook: Structure, Properties, Applications and their Nanocomposites: Wiley (2014) 2-13.
[2] LW. McKeen, Polyether Plastics. The Effect of Temperature and other Factors on Plastics and Elastomers (Third Edition), Oxford, (2014) 91-142.
[3] O. Olabisi, K. Adewale, Handbook of Thermoplastics, Second Edition: CRC Press, (2016).
[4] Y. Duan, H. Li et al., J. Appl. Polym. Sci. 99 (2006) 3085-3096.

ACRYLIC BONE CEMENTS MODIFIED WITH PEG/ALGINIC ACID SHAPE STABILIZED PHASE CHANGE MATERIALS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 57]

Introduction

Acrylic bone cement, based on poly(methyl methacrylate) (PMMA), is widely used for the fixation of artificial joints to the bone tissue but also in remodelling osteoporotic and vertebral fractures repair (1). It is usually supplied as a two component system: polymer powder and liquid monomer. Bone cement is prepared by mixing these two components and the liquid monomer polymerizes around the polymer powder microparticles to form hardened cement. In this free radical, exothermic polymerization process, huge amount of heat is generated to the environment. The temperature of bone cement during the curing process is in range from 40°C to 110°C (2). One of the method to reduce too high maximum polymerization temperature is using phase-change materials (PCM) e.g. based on poly(ethylene glycol) (PEG). PEG is very effective, biocompatible heat accumulator, but it needs to be stabilized to prevent the PEG leakage out of the system in the higher temperature. PEG can be easily stabilized with polysaccharides. In our preliminary study, we investigated the influence of pristine PEG of on the polymerization temperature of PMMA cement (3). The maximum temperature was reduced by 13°C, but the mechanical properties of cement decreased below requirements. Then, we decided to use potato starch as a shape stabilizer for PEG (4). The results confirmed that 15% of potato starch slightly reduced the thermal capacity of PEG but the mechanical strength of PMMA modified with PEG/potato starch systems was kept. In this study, PEG will be stabilized with different amount of alginic acid (AA) in order to investigate the influence of amount of polysaccharide on efficiency of PEG-based heat accumulators intended to PMMA cements.

Materials and Methods

Duracryl®Plus, an acrylic bone cement was purchased from Spofa Dental. PEG (Sigma - Aldrich) with average molecular weights of 4000, 8000 and 12,000 g/mol was used as a cement modifier. Alginic acid (AA) (Sigma - Aldrich) was added to the PEG as a shape stabilizer.

Preparation of PEG/AA systems

In the first stage, different amount of alginic acid (1g, 2g, 3g) was dissolved in 90 ml of 2% NaOH. Next, PEG of given molar mass was dissolved in the alginic acid solution in accordance with TABLE 1.

TABLE 1. PEG/AA systems composition.

AA / PEG:	10%	20%	30%
4000	9:1	8:2	7:3
8000	9:1	8:2	7:3
12000	9:1	8:2	7:3

The mixture was placed in a water bath at 72°C and stirred for 7 min. When the mixture was gelatinized, the solutions were poured into a Petri dishes and dried at room temperature for 7 days. Then, the blends were grinded finely in the mortar.

Acrylic bone cement was modified with 15% of PEG/AA systems. The liquid to powder ratio was 0.37ml/g. The curing temperature of cements was measured using an electronic thermometer in accordance with the ISO 5833 standard. Ultrasonic measurements and compression test, to estimate the mechanical properties of bone cement, were used. SEM analysis was used for the observation of surface morphology.

Results and Discussion

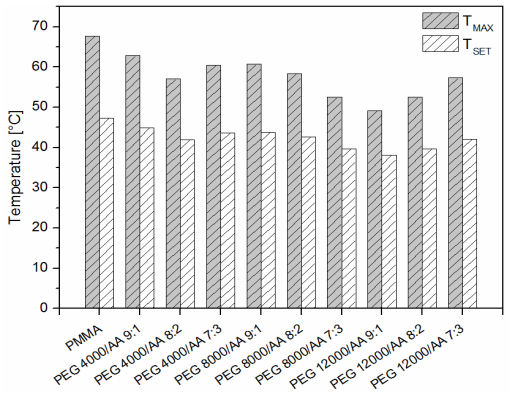

FIG. 1. The maximum temperature (T_{MAX}) and the setting temperature (T_{SET}) of PMMA/PEG/AA bone cements.

Temperature measurements (FIG. 1.) confirmed that all heat accumulators work effectively. The maximum temperature was decreased up to 17°C (PEG 12000/AA 9:1). The mechanical tests proved that the addition of stabilized PEG to the PMMA cement does not affect the mechanical strength significantly. SEM micrographs (FIG. 2) showed that the additive of PEG/AA system was evenly distributed on the PMMA surface.

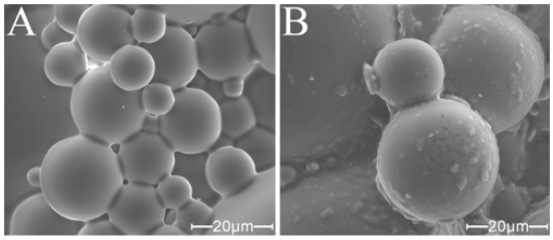

FIG. 2. SEM micrographs of PMMA (A) and PMMA/ PEG/AA system 9:1 (B).

Conclusions

In this work, acrylic bone cement was modified with 15% of PEG/AA shape stabilized PCM in order to reduce too high polymerization temperature of PMMA. Different molar mass of PEG and various AA content in heat accumulators were tested. It was proved that 20% of AA and PEG of 12000 are the most effective combination that allowed to decrease maximum temperature of cement and keep the mechanical properties of PMMA bone cement.

Acknowledgments

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

References

[1] R. Vaishya, M. Chauhan et.al. J. Clin. Orthop. Trauma. 4 (2013) 157-163.
[2] KD. Kuehn, W. Ege et al. Orthop. Cin. North Am. 36 (2005) 17-29.
[3] K. Król, K.Pielichowska, Polym. Adv. Technol. 27 (2016) 1284-1293.
[4] K.Król, B. Macherzyńska et al. J. Appl. Polym. Sci. 133 (2016) 43898-1-11.

CARBON NANOSCAFFOLDS FOR FIBROBLAST AND HEPATOCELLULAR CARCINOMA CELLS ADHESION, MIGRATION AND REGENERATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 58]

Introduction

As with many types of cancer, cell migration and adhesion is an important factor in the progression and metastasis of hepatocellular carcinoma (HCC) [1]. The carbon scaffold acts as an interim synthetic extracellular matrix (ECM) that cells interact prior to forming new tissue [2]. The scaffold should provide attachment, growth, differentiation of cell and must be porous for nutrients. The rate of degradation of the scaffold must be equal to the rate of tissue formation. The extracellular matrix and products of its degradation must be biocompatible. Strojny et al. [3] reported that diamond, graphene oxide and graphite are highly biocompatible and non-toxic for animals. The carbon nanoparticles are present for a long time after injection to tissue. This is the reason for their use as drug carriers [3]. In this study, human stromal cell line (HS-5) was used as control compared with neoplastic cells.

Material and methods

Carbon scaffolds were prepared by drops placement and desiccation of the colloids of nanoparticles of diamond (ND), fullerenes (F60), nanotubes (NT), nanotubes OH (NTOH), nanotubes COOH (NTCOOH), graphene oxide (GO), pristine graphene (GP) on the bottom of culture plates. HS-5 (ATCC, CRL-11882), HepG2 (ATCC HB-8065) and C3A (ATCC CRL-10741) were obtained from the American Type Culture Collection (ATCC). The human cell lines were maintained at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Medium-Low Glucose (DMEM, Gibco, Thermo Scientific, Waltham, MA, USA) supplemented with 10% Fetal Bovine Serum (Life Technologies, Houston, TX, USA), penicillin (100 U/mL) and streptomycin (100 mg/mL) (Life Technologies). Cells were seeded on 6-well plates containing 0.1% of carbon scaffolds. The cultures with scaffolds were maintained by one day. The location, density and agglomeration of cells on the scaffolds were examined by the inverted light microscope (Leica, TL-LED, Germany) connected to a digital camera (Leica MC190 HD), using LAS V4.10 software (Leica) and compared to the control. The mean viability of HS-5, HepG2 and C3A cells on carbon scaffolds was assessed by evaluation of metabolic activity, using the PrestoBlue (Life Technologies, USA) and XTT (Roche Protocol, Germany). Cell proliferation was evaluated using a bromodeoxyuridine (BrdU) incorporation assay (BrdU colorimetric) (Roche Applied Science, Indianapolis, IN, USA).

Results and discussion

Morphology, number and differentiation of cells depend on the type of nanoscaffolds and cell lines. The interaction of carbon allotropes with cells was different. All nanoparticles were non-toxic or slightly toxic to cells used. The scaffold, prepared from ND was the most colonised by HepG2 cells, moreover, stimulated cell proliferation (large agglomerations of cells on scaffolds). HS-5 and C3A cells were single on the diamond surface. Cell lines showed a high affinity to GO scaffold. HCC cells formed smaller aggregation on the GO surface, i.e. niche provides good growth conditions. Scaffold, prepared from fullerenes, was the least colonised by HCC cells and decreased the amount of cells. However, the number of fibroblast cells was high on the fullerenes scaffold and beyond it. The most neutral for used cells was pristine graphene. Cells showed higher affinity to small aggregations than to big aggregations of GP. Scaffold constructed by carbon nanotubes, functionalised with COOH, was also well settled by cells, better than scaffold with nanotubes and nanotubes OH. Microscopic observations have been confirmed by viability and proliferation assays.

Conclusions

Nano-scaffolds, depending on the carbon allotropes, influenced behaviour, regeneration and morphology of HS-5, HepG2 and C3A cells. Furthermore, cells changed bio-function of scaffolds. Carbon nanoparticles are biocompatible and have a favourable structure to colonization by specific cells. The scaffolds-cell interaction leads to adhesion and then affects the cell division. The worst niche for the cells used were the nanotubes, which resulted from the lack of suitable functional groups. Factors such as shape, atomic hybridization and proportion of chemical bonds influence behavior of cell lines.

Acknowledgments

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References

[1] C. Yang, M. Zeisberg *et al.,* Cancer Res. 63 (2003) 8312-8317.
[2] I.O. Smith, X.H. Liu *et al.*, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 1 (2009) 226-236.
[3] B. Strojny, N. Kurantowicz *et al.,* PLoS One. 10 (2015) 1-18.

CHITOSAN-BASED NANOCOMPOSITES MODIFIED WITH REDUCED GRAPHENE OXIDE PREPARED VIA GREEN SYNTHESIS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 59]

Introduction

Nanocarbon materials, such as graphene, graphene oxide (GO) and reduced graphene oxide (rGO) have shown great potential in biomedical applications [1]. Recently, particularly graphene modified nanocomposites became the subject of intensive research [2,3]. In this paper, we report simple method of GO reduction using green reducing agent – L-ascorbic acid and fabrication of chitosan based nanocomposites modified with GO, green-synthesized rGO and hydroxyapatite.

Materials and Methods

Graphene oxide was prepared from graphite by the modified Marcano method (ITME, Poland). Green synthesis of rGO was performed as follows: known amount of L-ascorbic acid (Avantor Performance Materials Poland S.A.) was added to 300 ml of aqueous GO dispersion (0.1 mg/ml) under vigorous stirring. Next, sodium hydroxide solution (1 M NaOH) was added drop-wise to adjust the pH of the suspension to 9-11. The whole system was then sonicated for 0.5 h and kept for 2h at 70 ^{0}C. To produce nanocomposites, dispersion of GO or rGO was added to 4.7% (w/v) solution of chitosan (Acros Organics, M=600000-800000) in 5% acetic acid and sonicated for 1h at RT. Homogeneous chitosan solutions modified with GO (1.5%), rGO (1.5%) or rGO and hydroxyapatite (6%; HA, Chema-Elektromet, Poland) were cast into Teflon dishes and left for 96h at RT.
Structural properties of the prepared nanocomposites were investigated by attenuated total reflection spectroscopy (ATR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). In addition, wettability and degradation behavior were investigated.

Results and Discussion

X-ray diffraction pattern of GO showed sharp peaks at 11.51^0 (d-spacing ~0.77 nm), which completely disappeared after the GO reduction and a new wide peak showed up at 22.83^0 (~0.39 nm). Reduction of functional groups in GO was confirmed by ATR spectroscopy - intensity of the oxygen groups peaks decreased significantly.
When added to chitosan matrix, GO flakes stacked together, while exfoliated rGO nanosheets aligned parallel to the film surface (FIG. 1). Chitosan/GO sample surface was relatively smooth (FIG. 1A). In comparison, chitosan/rGO (FIG. 1C) and chitosan/rGO/HA (FIG. 1D) samples had higher surface roughness with plenty of graphene flakes and hydroxyapatite particles visible. Modification of chitosan with GO and rGO resulted in decrease of water contact angle value (FIG. 2). In the case of GO modification, it can be mainly attributed to hydrophilic groups attached to nanosheets. Significantly reduction of contact angle was observed for both types of rGO-modified nanocomposites (i.e. chitosan/rGO and chitosan/rGO/HA), despite the smaller number of hydrophilic functional groups attached to rGO. It can be assumed that the surface roughness has a decisive influence on the wettability.

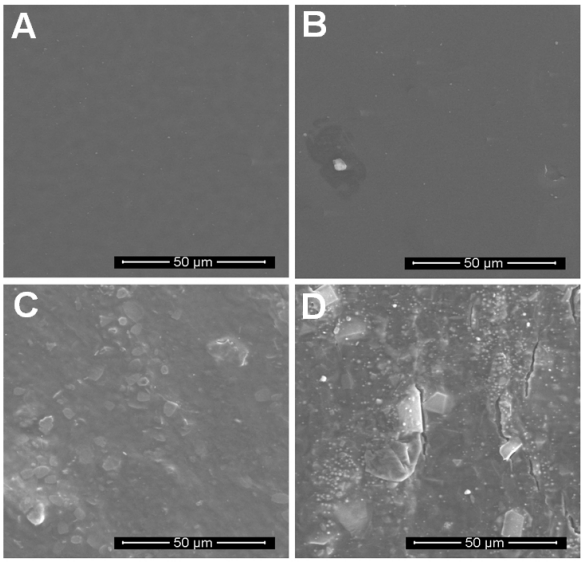

FIG. 1. SEM images of pure chitosan (A), chitosan/GO (B), chitosan/rGO (C), chitosan/rGO/HA (D).

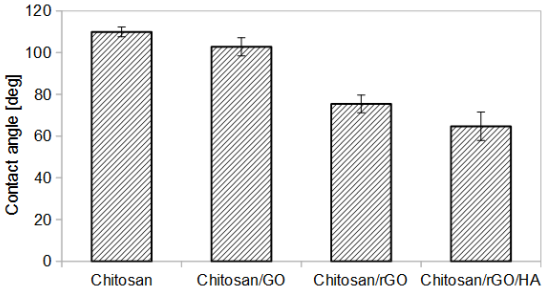

FIG. 2. Effect of nanofillers and HAP on contact angle.

Degradation behaviour of chitosan and its nanocomposites was investigated at 37^{o}C in three types of aqueous media: distilled water, PBS and Ringer's solution. Chitosan and chitosan/GO samples dissolved completely after only one day. The pH value decreased due to acetic acid residues and degradation products presence. Composites modified with rGO or rGO and HA were stable for weeks.

Conclusions

Reduced graphene oxide was successfully prepared via green synthesis using L-ascorbic acid as a reducing agent. Alkaline conditions provided colloidal stability of graphene oxide through electrostatic repulsion of the layers. Further, we have successfully prepared chitosan-based composites modified with GO, rGO and HA. Addition of green-synthesized rGO improved wettability and stability in PBS and Ringer's solution of the nanocomposite films. Both, chitosan/rGO and chitosan/rGO/HA are promising materials for biomedical applications, e.g. bone tissue engineering.

Acknowledgments

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References

[1] B. Zhang *et al*., Mater. Sci. Eng. C 61(2016) 953-964
[2] P. Yu *et al*., Carbohydr. Polym. 155 (2017) 507-515
[3] Y. Khan *et al*., Carbohydr. Polym. 146 (2016) 131-138

EFFECT OF ELECTROSPINNING CONDITIONS ON PLA FIBERS MORPHOLOGY AND UV DEGRADATION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 60]

Introduction

Nowadays, electrospinning (ES) is very popular method to produce fibers in micro- and nanoscale. Many of researches have focused on optimizing process conditions and obtaining fibers with properties optimal for biomedical application [1,2]. The most important parameters are: type of polymer, diameter and orientation of fibers, degradability [3] and bioactive substances addition.
The aim of the study was to determine effect of ES conditions on the properties of polylactide (PLA) fibers and investigate the effect of UV irradiation on fibers.

Materials and Methods

The process was performed using ES device dedicated to obtaining this kind of materials. In the process, PLA (NatureWorks LLC, USA) powder was dissolved in mixture of N,N-dimethylformamide (DMF, Avantor Performance Materials Poland S.A) and dichloromethane (DCM, Avantor Performance Materials Poland S.A.) with different ratios (1:3 and 1:2,5) and used as a raw material. The parameters of ES were optimized (temperature of process ~49°C and concentration of PLA (11-15%)).
Scanning electron microscope (SEM) was used to check diameter and orientation of fibers. The aging under UV irradiation process was examined in a specially designed chamber. The source of aging was the UV-C 11W bulb, and the ozone being the product of the decomposition of oxygen from the air.

Results and Discussion

SEM micrographics were used to obtain information about fibers diameters produced under different conditions. The results dependence of temperature is shown in FIG. 1. Most fibers had a diameter in the range of 100 to 500 nanometers. Higher temperature in ES chamber improves material quality. Fibers were more homogeneous, thinner and more repeatable.
In the next step, UV ageing chamber (UV-C 11W~ equal half year exposure sun) was used to investigate PLA fibers behaviour under UV/ O_3 conditions. The maximum exposure time was 24 h. After that time, the fiber microstructure was completely destroyed (FIG. 2).

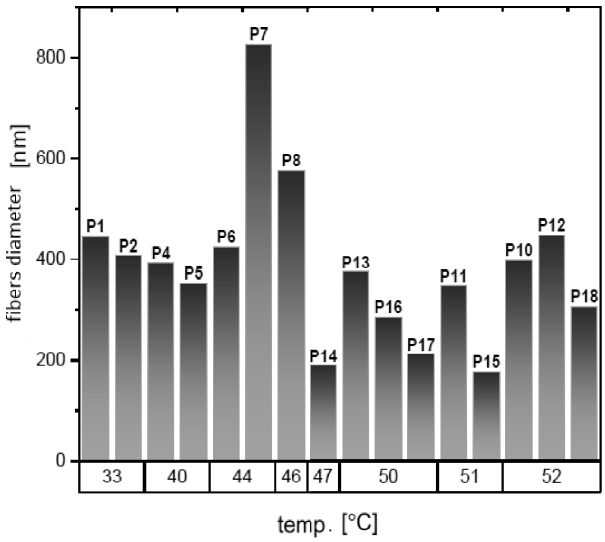

FIG. 1. PLA fibers diameters vs. temperature of ES process (P1-P18 number of sample).

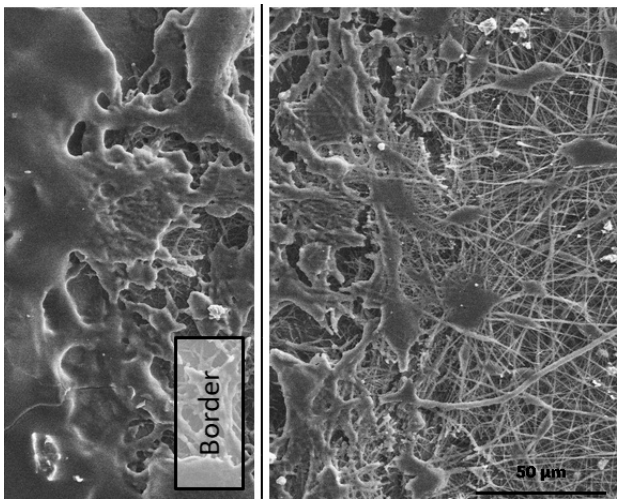

FIG. 2. Effect of UV and O_3 on the PLA fibers after 24 h exposition.

Conclusions

ES process is influenced by many factors: polymer concentration, temperature, type of solvent and solvents mixtures ratio. The results show that the best quality PLA fibers were obtained at a concentration of 13-15% and under temperature over the range 40-50°C.
PLA fibers are not resistant to UV irradiation. This is due to the chain build of the polymer. Chains under the influence of higher energy radiations are torn and split. This result in a significant weakening of the continuity of the layer composed of nano- and microfibers. PLA fiber materials for biomedical applications that may be exposed to sunlight (e.g. skin substitutes) have to be covered with a protective layer or contain UV-protecting substances.

Acknowledgments

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References

[1] J. R. Dias, P. L. Granja *et al.,* Prog. Mater. Sci. 84 (2016) 314-334
[2] B. Na *et al.,* Polymer 65 (2015) 55-62
[3] S. Lv *et al.,* Constr. Build. Mater. 144 (2017) 525-531

INVESTIGATION OF INITIAL DEGRADATION STAGE AND TENSILE STRENGTH OF POLYLACTIDE AND ITS COMPOSITES WITH EGGSHELLS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 61]

Introduction

Many research centres try to investigate new biocomposites for different purposes, contain materials from natural resources [1]. Polylactide (PLA) is a very popular biodegradable aliphatic polyester, which can act as an alternative to other polymers [2]. Its degradation rate depends on porosity, crystallinity, initial particle size and amount of additives [1]. What is more, bioadditives can also influence mechanical properties of polymer [3]. Natural bone is resistant to different stresses due to complex construction of inorganic and organic elements [4,5]. It inspires to design similar materials. The paper focuses on investigating tensile strength and the initial stage of degradation of polylactide and its composites with particles obtained from eggshells. Both of components are biodegradable, so testing their combination allows to consider the composite for application in an eco-friendly packing or medical material with mechanical properties comparable to natural [2,6,7]. Described tests introduce new results in the field of degradation and modification of polylactide with natural particles.

Materials and Methods

As a matrix of composites, technical polylactide unsuitable for natural body (Ingeo, Natureworks) was used. As a filler, particles of eggshells were prepared. Firstly, eggshells were washed, dried in an oven in 100ºC for 30 min and ground in electric grinder to the powder. Then they were stirred in 14.5% NaOH solution to remove organic parts, washed with distilled water, dried, then stirred in pure methanol for 30 min, decanted, washed with distilled water and dried. Polylactide was also dried in an oven in 50ºC to achieve constant weight. Three types of samples were prepared using Zamak Mercator injection machine: pure PLA, PLA with 10% eggshells [EG] and PLA with 20% of eggshells. Degradation of samples was tested in two different environments: distilled water and simulated body fluid (SBF), in 37ºC for a one month. Samples were mixed with solutions with mass ratio 1:10, each type separately. During this time, pH and electrolytic conductivity of solutions, in which samples were put, were investigated. What is more, tests were also performed for solutions without PLA and its composites in it. Mass of samples was tested regularly, both after soaking with solution and then drying it to constant mass, so solution absorptivity could be calculated. The surface of samples before and after degradation was observed using NOVA NANO SEM 200 microscope with EDS. Added eggshells were also investigated to determine their composition. Tensile strength and Young's modulus were tested on universal testing machine Zwick 1435, both before degradation and after one week.

Results and Discussion

FIG. 1. Samples after tensile test.

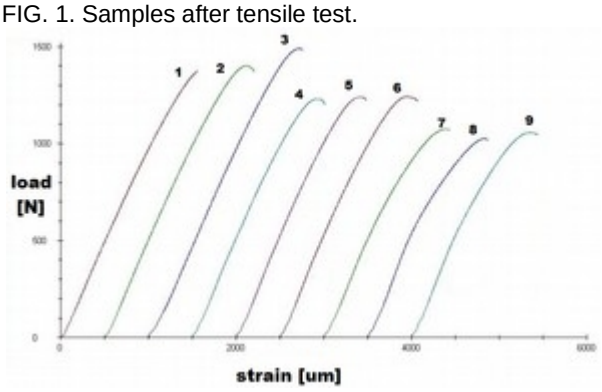

FIG. 2. Load-strain characteristics of tensile test for all types of samples (1-3: pure PLA, 4-6: PLA+10%EG, 7-9: PLA+20%EG).

From tensile test it could be observed that for non--degraded samples, Young's modulus increases and tensile strength decreases with the increase of the amount of EG additive. Absorptivity of solution by materials could be observed to a small extent. Conductivity and pH of solutions was changing irregularly differently according to the type of material. Observation on SEM with EDS of EG allowed to conclude about the influence of modification on it, and tested samples - about the influence on degradation on the surface.

Conclusions

Combination of PLA with particles of modified eggshells is a promising solution in the area of biodegradable composites. The advantage of this is availability of additive and interesting properties, which can be altered by changing the amount of EG.

Acknowledgments

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References

[1] J. Chłopek, A. Morawska-Chochół et al., Journal of Achievements in Materials and Manufacturing Engineering, 43 (2010) 72-79
[2] Y. Rudeekit, J. Numnoi et al., Journal of Metals, Materials and Minerals, 18 (2008) 83-87.
[3] L. Xiao, B. Wand et al., Biomedical Science, Engineering and Technology, ed. Prof. D. N. Ghista, 2012, www.intechopen.com
[4] A.D.P. Bankoff, Human Misculoskeletal Biomechanics, ed. dr. Tarun Goswami, 2012, www.intechopen.com
[5] T. Velnar, G. Bunc et al., Surgical Science, 6 (2015) 255-263
[6] B. J. Tiimob, S. Jeelani et al., Journal of Applied Polymer Science, 133 (2016) 431214
[7] A. Asha, V. C. Sekhar, International Journal of Engineering Research&Technology, 3 (2014) 288-291

POLYMERIZATION SHRINKAGE OF DENTAL COMPOSITES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 62]

Introduction

Dental composites are based on polymer resin matrix which diminishes its volume during polymerization process due to joining of monomer chains [1]. It is the reason of polymerization shrinkage of each polymer material. Serious consequence of the shrinkage in dentistry is marginal leakage and secondary caries resulting from this [2]. Therefore, the develop a low shrinkage material is a big challenge in the manufacturing of dental composites.

There are many methods of diminishing polymerization shrinkage. One group is focused on resin matrix composition, the second on filler selection [3] and the others on applying technique [4].

Literature presents a lot of methods of shrinkage measurements [1]. In previous study the Authors used the method based on microCT measurements [5]. In this paper a new approach has been presented.

In this study, the new method of polymerization shrinkage was applied to evaluate the polymerization shrinkage of selected dental composites showing differences in composition.

Materials and Methods

Materials used in this study were:

- Flow-Art (Arkona), 38% wt. of resin mix: Bis-GMA, UDMA, TEGDMA and Bis-EMA) and 60% wt. of fillers (Ba-Al-Si glass and nanosilica);
- Boston (Arkona), consist of 20% resin mix: Bis-GMA, UDMA, Tri-EDMA (TEGDMA), EBADMA and about 78% wt. of fillers: Ba-Al-Si glass, pyrogenic silica;
- Charisma Opal Flow (Heraeus), which was composed of Bis-GMA resin and about 58% wt. of fine inorganic fillers (BA-Al glass and silica).

Each material had the shade of A2.

Volumetric shrinkage measurements was conducted using microCT Skyscan 1174 (Bruker microCT) with accuracy of 6.5 μm. Volume of composite's drop was measured assuming it is a body of revolution, formed by rotation of half of its cross-section. A drop of composite (volume of about 3 mm^3) was placed on tip made of PE (d = 3 mm). After 3 minutes time (material's spreading) 5 images were taken in different angle position (0, 45, 90, 135 and 180°). In next step composite was cured using Cromalux 75 halogen lamp with special limiter (FIG. 1). After curing and additional time of 1 min (dark polymerization [6]) another set of 5 images were taken in appropriate angular position. Override of images taken before and after curing is presented on FIG. 2. Dark line in upper region means the difference in volumes. Results were statistically analyzed using Statistica ver. 13 software (Dell Inc. 2016).

Results and Discussion

Results of measurements are presented on FIG. 3. Highest value of volumetric shrinkage (3.70% ±0.70) was observed for FlowART composite. The same resin and different volume of filler has the Boston composite. Its shrinkage was significantly lower (2.44% ±0.16). The Charisma Opal Flow composite has almost the same ratio of components as Flow-Art, but different resin was used. In this case the shrinkage had a value of 2.86% ±0.30. It is noteworthy that spread of all results (S.D.) was very low, which testify the quality of measurements. All results were statistically different on significance level α = 0.05.

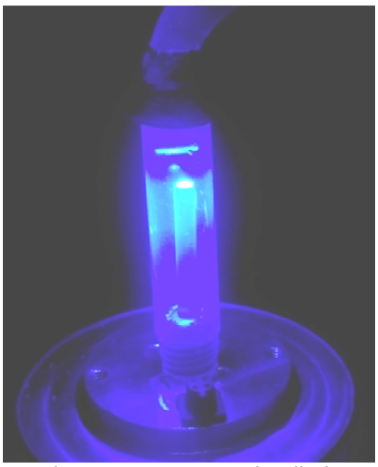

FIG. 1. Light curing on CT stage using limiter.

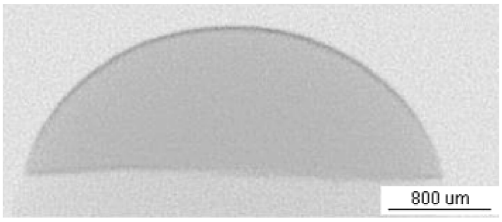

FIG. 2. Change of the volume due to polymerization shrinkage.

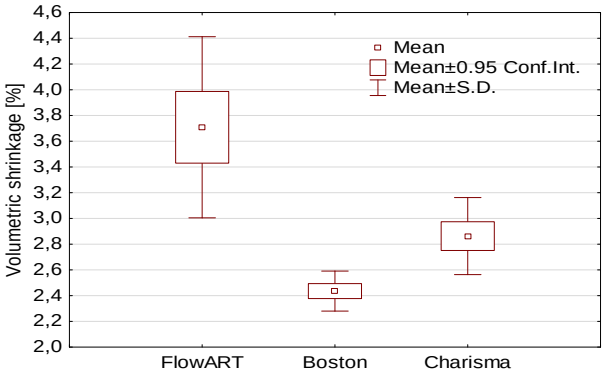

FIG. 3. Volumetric shrinkage of tested materials.

Conclusions

All tested materials showed low value of polymerization shrinkage, comparable with other commercial dental composites. The influence of material composition on polymerization shrinkage was demonstrated.

Acknowledgments

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References

[1] Lee L.B., Min S.H. Dent Mater. 28 (2012), 212-8
[2] Patodiya A., Hegde M. National Journal of Community Medicine, 3 (2012), 754-756
[3] Chung C., Dent Mater, 18 (2002), 174-178
[4] Yoshikawa T. Dent. Mater, 17 (2001), 359-366
[5] Pałka K. et al. Engineering of Biomaterials / Inżynieria Biomateriałów 75 (2016), 75

ENDOTHELISATION OF DECELLULARIZED PERICARDIUM WITH HEPARINIZED FIBRIN COATINGS IN IN-VITRO BIOREACTOR

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[ENGINEERING OF BIOMATERIALS 143 (2017) 63]

Introduction
Decellularized matrices hold a great promise in advanced tissue engineering and repair of irreversibly damaged tissues in cardiovascular surgery. A cross-linked xenopericardium is commonly used as a patch in cardiac surgery but it didn't facilitate cell ingrowth and remodelling. Coating these matrices with autologous fibrin with covalently attached heparin and grow factors (FGF-1, FGF-2, VEGF) can minimize the thrombogenicity and can act as attractants to promote spontaneous endothelisation. *In-vitro* simulation of physiological conditions like those in blood vessels creates a tool for optimizing these coatings and their translation in to *in-vivo* experiments.

Materials and Methods
There are three possible ways of endothelization of patches in body: trans-anastomotic, trans-mural and blood/bone marrow-derived. For simulating these physiological conditions i*n-vitro* a special cultivation chamber with computer controlled perfusion system was created. The cultivation chamber allows fixing decellularized pericardium tissue and creates two compartments on each side of tissue. In this chamber the decellularized pericardium is coated with fibrin (with heparin and/or grow factors). Each side of pericardium can have different coating or different culture medium to creating concentration gradients. The endothelial cells (HUVEC) or stem cells (ASC) in suspension are seeded in thin strip shape on pericardium. Coating and seeding is done via sterile septum. The perfusion system creates two types of physiological stimuli simulating conditions in blood vessels. The controlled flow generates shear stress in physiological range. The pressure stimulation creates pulsatile mechanical loading.
After initial adhesion of the cells the perfusion system is activated to create dynamic physiological conditions. After defined period (7 to 21 days) the tissue removed from chamber. This tissue is histologically evaluated to get information about cell migration and proliferation and their ability to in grow into tissue based on the coatings and grow factor concentration.

Results and Discussion
Coatings of pericardium with fibrin and with covalently attached heparin and grow factors improve the cell proliferation and their migration over scaffold in contrast to only decellularized pericardium. The optimal concentration of grow factors must set based on further analysis. Also, dynamic cultivation, unlike static, provides better response of cells e.g. their orientation and morphology caused by flow and mechanical loading simulating more *in-vivo* like conditions.

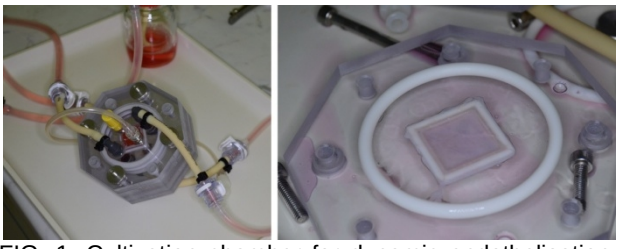

FIG. 1. Cultivation chamber for dynamic endothelisation of decellularized pericardium connected to perfusion system and sterile ports (left), fixed decellularized tissue in chamber (right).

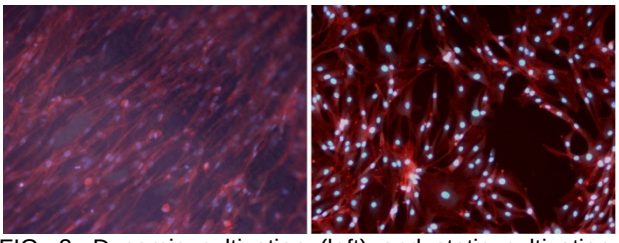

FIG. 2. Dynamic cultivation (left) and static cultivation (right).

Conclusions
Heparinized fibrin coatings with grow factors on decellularized pericardium promoted its endothelisation. Designed *in-vitro* bioreactor provides tool for optimizing these coatings and their translation to *in-vivo* experiments.

Acknowledgments
This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-29153A and by BIOCEV – "Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" project (CZ.1.05/1.1.00/02.0109), funded by the European Regional Development Fund

References
[1] Sakiyama-Elbert SE and Hubbell JA. Development of fibrin derivatives for controlled release of heparin binding growth factors. J. Control. Release 2000; 65:389-402
[2] Zisch, A.H., et al. Covalently conjugated VEGF-fibrin matrices for endothelialization J Control Release.2001; 72:101-113.
[3] Kaplan O., Zarubova J., Mikulova B., Filova E., Bartova J., Bacakova L., Brynda E. Enhanced mitogenic activity of recombinant human vascular endothelial growth factor VEGF121 expressed in E. coli Origami B (DE3) with molecular chaperones (2016) PLoS ONE, 11 (10)

RESPONSE OF ASC CELLS TO PRESSURE STIMULATION ON OXYGEN TERMINATED NANOCRYSTALLINE DIAMOND SURFACE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 64]

Introduction

One of the methods utilized in the treatment of irreparable damage to bone is bone replacement by synthetic implant. It is usually made from metal, ceramic or combination thereof. After implantation into the patient together materials and living tissues interact [1]. The reaction is dependent on the physicochemical properties of the material, which may cause various cell responses, thus affecting the adhesion of cells to the implant [2]. To eliminate problems in the interaction of the implant with the surrounding tissue implant surface is modified by applying different surface layers. Appropriate modification of the surface can modify the functional interface bone – implant – it means increasing of osteointegration, reducing the healing time and allowing for early loading of the implant [3]. The starting point of the above disadvantages is covering of the implant surface by autologous cells of the patient. Many studies showed that static cultivation supports insufficiently osteoblast differentiation of stem cells [4]. Dynamic cultivation with defined stimulation can improve this process and creates more *in-vivo* like conditions.

Materials and Methods

The aim of this study was to achieve response of adipose stem cells (ASC) on nanocrystaline diamond surface NCD under pressure stimulation in vitro. A custom-built bioreactor was created. This system consists of the specially designed cultivation chamber and pressure generating linear pump. The cultivation chamber allows fixing NCD substrate and creates reservoir for culture medium. The cells are seeded there over sterile septum. The overall construction was optimized for time-lapse microscopic imaging. Computer controlled custom-built linear pump with pressure sensing is used as pressure generator. This solution allows setting pressure range up to 700 mmHg and frequency up to 2 Hz.

The substrate both for dynamic and static cultivation was tissue treated glass (dimensions 24x24 mm, 0.15 mm thickness) with deposited a thin layer of NCD. The thickness of NCD was approximately 200 nm (depending on the length of the deposition). This layer was in the last step of deposition terminated with oxygen, resulting in a high surface wettability.

The substrates were seeded with density of 70 000 cells/cm^2. This density was set to get nearly confluent coating. After 1 hour a pulsatile cyclic strain of 50/100 mmHg, at a frequency of 0.2 Hz was set for 71 hours. The static control was left without stimulation. The fresh DMEM culture medium supplemented with 10% FBS, 50 ug/ml ascorbic acid, 10 mM B-glycerophosphate, 10 nM dexamethasone was used. After 72 hours, the substrates were removed and the cells were fixed and fluorescently stained for further analysis.

Results and Discussion

There was significant difference in morphology between dynamic and static cultivated cells observed by light microscopy. Analysis of alkaline phosphatase, collagen-1 and osteopontin using fluorescent staining observed that stimulated cells express scientifically more genes than static cultured cells. These 72hours experiments were aimed at verifying the hypothesis of advantages of dynamic stimulation and testing of stimulating system. In the future experiments with longer duration are supposed to achieve differentiation of stem cells into osteoblasts.

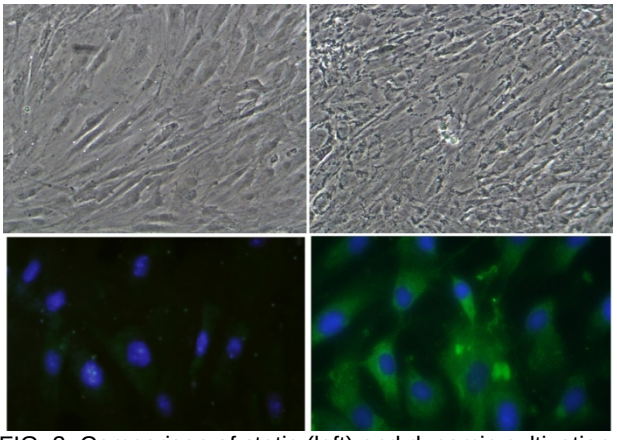

FIG. 2. Comparison of static (left) and dynamic cultivation (right), in phase contrast microscopy (top) and collagen-1 fluorescence staining (bottom).

Conclusions

Dynamic cultivation with pressure stimulation of ASC cells on O_2-terminated nanocrystaline diamond substrates has influence of cell proliferation and supports production of early markers of osteogenic differentiation in contrast to static cultivation.

Acknowledgments

This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-33018A and by BIOCEV – "Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" project (CZ.1.05/1.1.00/02.0109), funded by the European Regional Development Fund

References

[1] Okumura, Akira, Masaaki Goto et al. Substrate affects the initial attachment and subsequent behavior of human osteoblastic cells (Saos-2). Biomaterials. 2001, 22(16), 2263-2271.

[2] Eastlund, T. Infectious disease transmission through cell, tissue, and organ transplantation: Reducing the risk through donor selection. Cell Transplantation. 1995, 4(5), 455-477.

[3] Liu, X, P Chu a C Ding. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Materials Science and Engineering: R: Reports. 2004, 47(3-4), 49-121.

[4] Boris Antonov et al. Porous Coated Titanium Implants do Not Inhibit Mesenchimal Stem Cells Proliferation and Osteogenic Differentiation, Biotechnology & Biotechnological Equipment. 2013. 27:6, 4290-4293.

Ti6Al7Nb ALLOY SURFACE IMPROVEMENT FOR IMPLANTABLE BLOOD PUMPS APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 65]

Introduction

Despite the significant advances in continuous flow heart assist devices technology, these pumps have been associated with important adverse events, including pump thrombosis, stroke, bleeding, and device-related infection, and have caused unanticipated alterations in human biology that are related in part to shear stresses of the pump or reduced pulsatility [1,2]. Construction of implantable blood pump is a huge challenge in the aspect of long-term contact with blood under high shear stress conditions.

In the clinical version of Polish implantable rotary blood pump (FIG. 1) ReligaHeart ROT (RH ROT) [3] the polymer parts has been replaced with ceramic composite, ZrO_2-Y_2O_3, with high hardness, to improve device wear resistance. Additionally, modification of the well-known glow discharge assisted nitriding process called active screen plasma nitriding has been used for enhancing the properties of titanium pump parts, made from Ti6Al7Nb alloy through production of TiN+Ti_2N+αTi(N) diffusive surface layers in order to increase corrosion and wear resistance as well as device's biocompatibility.

FIG. 1. ReligaHeart ROT implantable blood pump clinical version prototype.

Materials and Methods

The athrombogenic diffusive nitrided surface layers TiN+Ti_2N+αTi(N)- type have been produced on Ti6Al7Nb titanium alloy surface, with roughness of Ra=80nm, using plasma nitriding process with active screen.

The microstructure and surface topography of the TiN – outer zone of nitrided layer were examined using TEM, SEM and AFM. HV0.05 Vickers microhardness measurements were carried out. Corrosion resistance, including impedance and potentiodynamic methods, as well as wear resistance (PN-83/H-04302 and ASTM G99-05 standards), of TiN layers were studied.

First investigation of TiN biocompatibility properties were performed according to PN EN ISO 10993 standard requirements. Biomaterials were sterilized with ETO, as the final device RH ROT sterilisation method (EOGas 4, H.W.Andersen Products Ltd.). Haemolysis assessment was performed on diluted human blood (HGB 10g/L) in direct contact. Biomaterial was incubated with blood in temperature of 37°C for 3h. After incubation free haemoglobin level was assessed and haemolytic index was calculated. Cytotoxicity examination was 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. Biodegradation tests were carried out for 30 and 60 days in SBF. The degradation medium was investigated by ICP analysis. Biomaterial surface morphology was investigated by SEM. Thrombogenicity assessment was carried out in static as well as in dynamic conditions. Static thrombogenesis evaluation was performed by biomaterial incubation in platelet rich plasma, temperature of 37ºC for 1h, washing and fixation with formaldehyde. Presence of adhered blood elements on biomaterials surface was investigated with SEM utilisation. Evaluation of thrombogenic properties in dynamic conditions was performed utilizing Impact-R analyser and fresh human blood, by generating physiological blood flow above the investigated surface. Platelets activation and platelet-leukocyte aggregates formation were determined utilizing flow cytometry. Analysis of adhered cells to the biomaterial surface with active receptors (CD62P, CD45) was performed utilizing fluorescent microscopy.

Results and Discussion

TiN+Ti_2N+αTi(N) layers produced on Ti6Al7Nb using glow discharge nitriding process with active screen improve titanium alloy surfaces hardness (FIG. 2), wear (FIG. 3) and corrosion resistance, allow to confirm the homogeneity of surface layer on the whole biomaterial samples area.

The first results of biological evaluations have confirmed that TiN+Ti_2N+αTi(N) layers produced on Ti6Al7Nb titanium alloy surface are non- haemolytic and non-cytotoxic with additionally high resistant for biodegradation and are characterized by good athrombogenic properties.

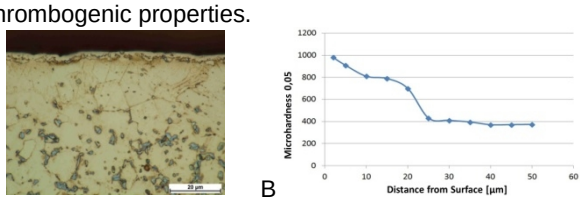

FIG. 2. Microstructure (A) and microhardness profile (B) of TiN+Ti_2N+αTi(N) layers produced on Ti6Al7Nb alloy.

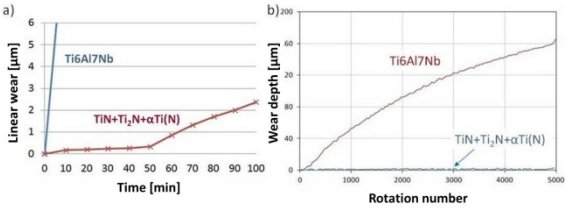

FIG. 3. Wear resistance of TiN+Ti_2N+αTi(N) layers and initial state Ti6Al7Nb alloy.

Conclusions

The TiN+Ti_2N+αTi(N) nitrided layers, with nano-crystalline TiN outer zone, produced on Ti6Al7Nb titanium alloy using active screen plasma nitriding process will improve ReligaHeart ROT parts hardness, wear and corrosion resistance, also in contact with zirconia parts during blood pump working. TiN layers have necessary biological properties; however complex biocompatibility has to be evaluated in order for biomaterial application in finale medical device for human use.

Acknowledgments

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

References

[1] F. Paganini *et al*., Journal of Thoracic Cardiovascular Surgery 151 (2016):10-2
[2] R. Starling *et al*., The New England Journal of Medicine, 380 (2014):33-40
[3] R. Kustosz *et al*., Archives of Metallurgy and Materials, V.60, 3/2015: 2253–2260

POLYURETHANE FILTRATION AS AN EFFECTIVE METHOD OF MATERIAL CLEANLINESS IMPROVEMENT FOR IMPLANT APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 66]

Introduction

Polyurethanes (PUs) are a gold standard biomaterial for medical devices applications due to its good biocompatibility [1]. Different PUs are used in pulsatile cardiac assist devices and total artificial heart prostheses (FIG. 1) for long-term utilization up to few years [2-4].

A B C

FIG.1. Pulsatile heart prostheses: A - BerlinHeart EXCOR [3], B – ReligaHeart EXT [2], C – CARMAT TAH [4].

Irrespective of detail heart prostheses design, the main parts of pulsatile prostheses made from PUs are elastic membranes responsible for blood flow in the prosthesis. The membranes have usually thickness below 1 mm and make analogical work to heart muscle, pumping from 40 to 80l of blood in every cycle, with frequency from 40 to 120 beats per minute. Therefore the elements should have extremely wear resistance for multiply work up to few millions cycles in biological active blood environment. PU membranes freedom of any contaminations and inclusions is one of the most relevant factor in order to confirm safety and reliability of the final device. The double filtration method was developed for PU solution cleanliness improvement for clinical utilization.

Materials and Methods

PU ChronoFlex AR/LT (AdvanSource Bomaterials, USA) is used for thin membranes manufacturing by dipping technology. Silica particles were observed in PU, derived from nano-silica aggregates added to the material during its manufacturing process. Size of inclusions observed in PU had been unacceptable for implant manufacturing, therefore the special double filtration process was developed in order to improve PU purity. The filtration process was performed in clean-room area in closed technological stand (tight cabinet) in argon atmosphere, with stain-less steel disposable removable filtration different sizes mesh utilization.
Thin foils (0.4 mm) of ChronoFlex AR/LT before and after double filtration process were examined. The following material properties were tested before and after filtration: viscosity (HAAKE Viscometer E), solid content, molar mass using GPC (DAWN HELEOS Wyatt Technologies and RI detection WGE Dr. Bures) and glass temperature using DSC (TA Instruments DSC2010) as well as mechanical properties - tensile strength, stress at break, elongation at max. tensile stress and elongation at break (according to PN-EN ISO 527-1:1998 and PN-EN ISO 527-3:1998). Biomaterial inclusions were investigated with KEYENCE Digital Microscope VHX-5000 utilization.

Results and Discussion

The molar mass and molar mass dispersion index of PUs before and after filtration process have not changed (FIGs. 2, 3). Glass temperature, mechanical properties, viscosity and solid content of ChronoFlex AR/LT do not differ before and after the filtration process (TABLE 1). Microscopic observation showed less inclusions in PU after filtration, in the thin foils randomly selected areas (FIG. 4).

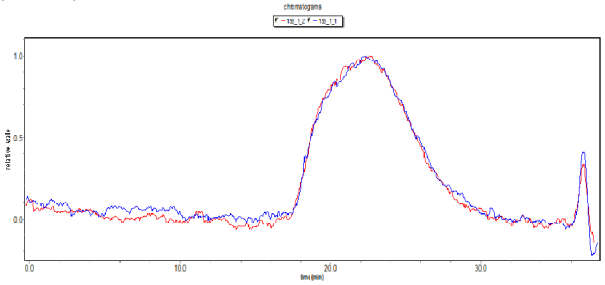


FIG. 2. GPC analysis of non-filtrated PU.

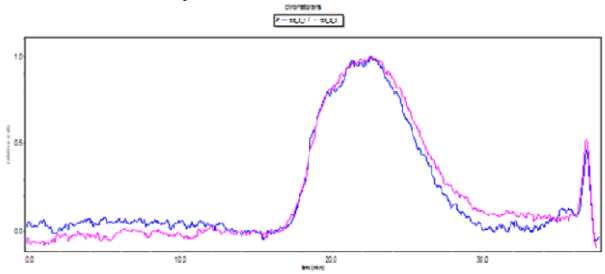
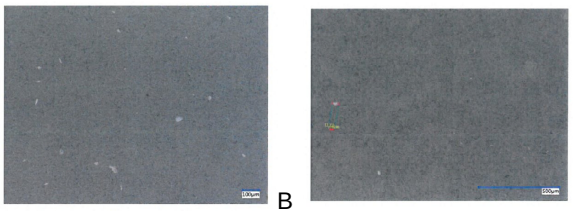


FIG. 3. GPC analysis of PU after filtration.

TABLE 1. Mechanical properties.

No.	TEST	Not filtrated material	Filtrated material
1	Tensile strength [MPa]	53.7	54.47
2	Stress at break [MPa]	53.7	54.46
3	Elongation at max. tensile stress [%]	951.18	929.06
4	Elongation at break [%]	951.22	929.16
5	Viscosity [cps] 30 400	30 410	30 390
6	Solid cont. [%] 23.06	23.04	23.05



A B

FIG. 4. PU inclusions: A before filtration, B after filtration

Conclusions

The results confirmed the new developed double filtration process of PU ChronoFlex decrease number of inclusions not changing PU mechanical and physical properties.
The filtration process final efficiency was validated after quality control of thin membranes produced from filtrated PU, showing lower number of inclusions in the final product.

References

[1] Wang W. & Wang C. In The Design and Manufacture of Medical Devices, Elsevier Ltd. (2012): 115-151
[2] M. Gonsior *et al.*, Archives of metallurgy and materials, V.60, 3/2015: 2271 – 2278
[3] www.berlinheart.de
[4] www.carmatsa.com

INITIAL BIOCOMPATIBILITY ASSESSMENT OF CERAMIC MATERIAL INTENDED FOR APPLICATION IN IMPLANTABLE HEART ASSIST DEVICE

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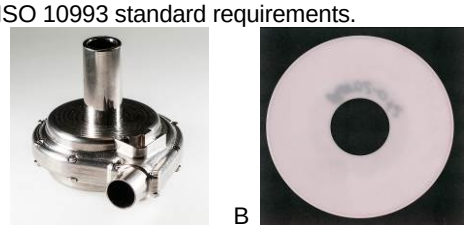
[ENGINEERING OF BIOMATERIALS 143 (2017) 67]

Introduction

Construction of an implantable continuous flow ventricular heart assist devices (VADs) requires detailed material selection in the aspects of long-term contact with blood under high shear stress conditions. Materials for VADs application should characterise high mechanical resistance and biocompatibility in order to reduce platelet activation on blood contacting pump elements and avoid blood clothing inside the pump. The centrifugal continuous flow blood pump ReligaHeart ROT (RH ROT), with contactless magnetically and hydro-dynamically suspended rotor has been developed as an implantable VAD for advanced heart failure treatment support (FIG. 1A) [1]. The blood pump dielectric elements made of zirconia stabilized with yttrium ZrO_2-Y_2O_3 [2] were evaluated in the aspect of long-term blood contact application in RH ROT elements (FIG.1B). The initial biocompatibility assessment of zirconia included biodegradation, haemolysis, cytotoxicity and thrombogenic properties analysis were performed in accordance with PN EN ISO 10993 standard requirements.



A B

FIG. 1. ReligaHeart ROT implantable clinical version prototype (A), dielectric ceramic elements (B).

Materials and Methods

Zirconia samples were prepared in a representative manner for the medical device.

Degradation evaluation

Degradation analysis was performed according to ISO 10993-14 standard at temperature of 37°C for period of 120 ±1h, in vitro in Tris-HCl buffer. Biodegradation tests were performed in SBF. Incubation was carried out for 30 and 60 days. After degradation process the medium was analysed by Inductively Coupled Plasma (ICP) for zirconia migration evaluation. Morphological surface changes after degradation process were investigated with SEM and SEM-EDX utilization.

Cytotoxicity evaluation

Cytotoxicity test was performed in accordance with PN EN ISO 10933-5 by qualitative method with utilization of normal mouse fibroblasts - NCTC clone 929.

Haemolysis evaluation

Haemolysis assessment was performed on whole human blood, CPDA-1 preserved, in direct contact and static conditions. The blood was diluted with PBS to haemoglobin concentration of 10 g/L. Before examination, initial blood count and free haemoglobin level (fHGB) in blood plasma were assessed. The investigated material was incubated with blood in temperature of 37^{o}C for 3h. Additionally, a blank test was performed under conditions given in test procedure. The free haemoglobin level was assessed and haemolytic index was calculated for blood, after incubation.

Thrombogenicity evaluation

Thrombogenicity assessment was carried out in static as well as in dynamic conditions. Static thrombogenesis evaluation was performed by incubation of the investigated material in platelet rich plasma in temperature of 37^{o}C for 1h. After incubation the investigated material was washed and fixed with formaldehyde. Presence of adhered blood elements was investigated using SEM. Evaluation of thrombogenic properties in dynamic conditions was performed utilizing Impact-R analyser and fresh human blood. The test consist of generating physiological blood flow above the investigated surface. Afterwards platelets activation and platelet-leukocyte aggregates formation was determinate utilizing flow cytometry. Analysis of adhered cells to the biomaterial surface with active receptors (CD62P, CD45) utilizing fluorescent microscopy was performed. Bionate (DSM) polyurethane was investigated as it has a low thrombogenicity potential and polystyrene as a reference material.

Results and Discussion

The results of the degradation tests of ZrO_2-Y_2O_3 showed material stability during degradation process.

Cytotoxicity analysis revealed no adverse effects of zirconia on fibroblasts. Cells characterized normal viability and proliferation.

The haemolysis index of the blood after contact with the investigated material was below 2% (classification of non-haemolytic material according to ASTM F756-00 standard).

The microscopic analysis of surface, after contact with blood in static conditions, revealed proper athrombogenic material properties (low both: platelets adhesion and activation). The number of activated platelets (CD62P) and aggregates platelet-leukocyte (CD62P-CD45) in blood circulating above the biomaterials surface was similar in all tested groups. The number of platelets (CD62P) and platelet-leukocyte aggregates (CD62P-CD45) adhered to investigated biomaterial surface were slightly lower than the number of activated elements on polystyrene surface. The number of adhered platelets (CD62) and aggregates in other test groups was comparable.

Conclusions

Primary investigation's results revealed high biocompatibility properties of zirconia stabilized with yttrium. The investigated material can be classified as non-degradable, non-cytotoxic, no-haemolytic and low thrombogenic.

The research will be continued in order to perform in vivo biocompatibility evaluation of ZrO_2-Y_2O_3 in the aspect of long-term contact with human body in RH ROT device implementation.

Acknowledgments

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

References

[1] R. Kustosz *et al*., Archives of metallurgy and materials, V.60, 3/2015: 2253–2260
[2] Piconi C. *et al*. (1999) V.20 I.1 :1-25
[3] R. C. Garvie *et al*. (1984) Journal of Material Science 19:3224-3228
[4] J. Li *et al*. (199) Clinical materials;12(4):197-201

MECHANICAL DURABILITY OF TWO EXTERNAL FIXATOR CLAMPS FOR JAM FIXATOR IN SMALL ANIMALS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 68]

Introduction

External skeletal fixation has become a well established treatment modality for many traumatic and degenerative orthopaedic defects in dogs and cats. During the last decade many of the significant developments in external skeletal fixation has been introduced [1-2]. The changes including improvements in implants, new materials for fixator elements and new fixators based on high technology. The JAM external fixator is one of the most popular fixator for fracture treatment in small animal orthopaedics. It is simple, but versatile modular construction, which allowed to construct complex frames for fracture treatment, traumatic luxation, arthrodesis and other diseases where the multiplanar configuration of fixator is necessary. The JAM fixation clamps have a two-piece clamp body, which allowed to remove or add fixation clamp from the frame at any time [3].
The paper present the biomechanical study of two different clamps of JAM (Meynard) fixator.Two external stabilizers have been investigated for their mechanical durability and suitability in orthopaedic surgery of small animals. Two JAM Meyerd stabilizers were compered the first with standard steel clamps and the second with polymer clamps (polyamide 66, PA 66). It has been shown that in spite of poor mechanical performance, polymer stabilizer may be an alternative to conventional steel clamps.

Materials and Methods

Meynard type stabilizer clamps made of medical grade stainless steel (316L) and polyamide (PA66 Termoplastik) clamps (supports/screws) were tested. Mechanical testing of the stabilizer was performed on a model system during cyclic axial load testing (stretching and compression) of the device. Cycles illustrated the effects of loading bone during real convalescence. Three basic parameters were analysed:
- plastic deformation energy (dissipate energy) (ΔW_{pl}) - determined surface area of the hysteresis loop recorded for a single cycle,
- difference in plastic elongation (ΔL) - read for a force equal to 0, for three random loops from each turn,
- maximum strain (F_{max}) readings for single hysteresis in load cycle.
All tests were carried out on the universal machine Zwick 1435.Matlabsoftware was used to analyse the mechanical results (bubble test for data sorting). Origin software for mathematical data processing was used.

Results and Discussion

All mechanical results are presented in TABLE 1. It's shown that better mechanical parameters then traditional stainless steel clamps. Fixator with polymer clamps characterised higher elasticity and lower stiffnes. This means that the fixator with PA66 will maintain the specified gap to an accuracy of 0.3mm for shorter time than the steel fixator. The indicated differences do not significantly affect the quality of the machine throughout the fatigue test (1000 cycles under the same conditions). Higher system flexibility and lower stiffness of the stabilizer with polyamide clamps compared to the steel stabilizer are limits the number of applications. However, due to its lighter construction and X-ray transparency in contact with PA66, it is an alternative to steel fixators used for osteosynthesis (FIG. 1).

TABLE 1. Summary of experimental results of mechanical tests.

	mass, g	plastic deformation energy, mJ	difference of plastic elongation, mm	maximal strain for maximum elongation, N
steel clamps (316L)	12.5± 0.19	4.8±0.22	0.0708	44.9±1.84
polymer clamps (PA66)	4.2± 0.05	7.6±014	0.218	30.6±2.56

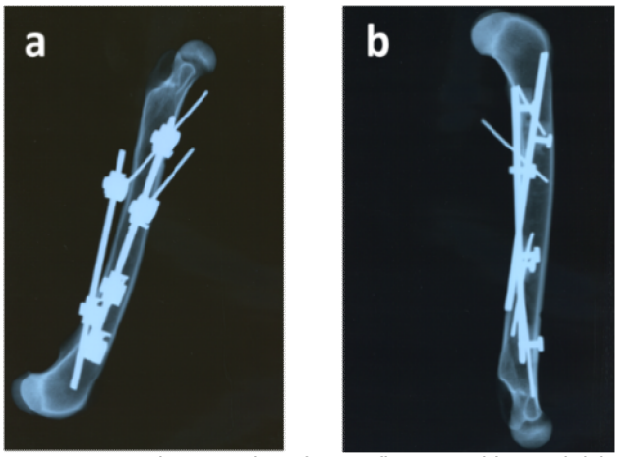

FIG. 1. RTG photography of JAM fixators with steel (a) and polymer clamps (b) fixed in rabbit bones.

Conclusions

As many fixators, which elements was made of steel, JAM fixator is heavy, particularly when is used for small animals and the radiographic assessment of bone is impaired due to metal component on radiographs. To eliminate this drawback, for clamp construction we used the Polyamide PA66, which weigh less than comparable steel clamps and is radiolucent.

Acknowledgments

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References

[1] D. Lewis *et al* J Small Anim Pract, 42 (2001) 103-109.
[2] K. Kraus *et al* Vet Surg, 27 (1998) 224-230.
[3] R. Gilley *et al* Vet Surg, 30 (2001) 341-350.

SILVER NANOPARTICLES FOR MEDICAL APPLICATIONS PRODUCED WITH THE USE OF STABILIZING-REDUCING COMPOUNDS DERIVED FROM NATURAL RESOURCES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 69]

Introduction

Silver nanoparticles (SNPs), are among the most attractive nanomaterials, and thanks to their antibacterial properties they have been widely used in a range of biomedical applications, e.g. in medical device coating, dental instruments, bandages and for personal health care [1,2]. They have also found applications in the diagnosis and treatment of cancer and as drug carriers in eye care for coating contact lenses [3]. In addition, the use of SNPs in combination with vanadium oxide in battery cell components was found to improve battery performance in the next-generation active implantable medical devices. SNPs can be used in bio-diagnosis, where their plasmonic properties strongly depend on size, shape and dielectric properties of the surrounding medium [4]. A wide range of physical and chemical techniques have been developed to produce SNPs of different size, shape and compositions [3,5]. Nowadays pioneering, more environment-friendly techniques that are using biological substrates are of particular importance [6].
The aim of this study was to obtain SNPs suspension with desirable physicochemical characteristics with the use of extracts of different kinds of coffees acting as stabilizing-reducing agents.

Materials and Methods

Samples of coffees from around the world were tested as reducing and stabilizing agents in chemical reduction method of SNPs preparation processes. Nanosuspension was obtained by adding 0.07875 g of $AgNO_3$ salt to 100 ml of water and dropwise 7 ml of the cooled coffee extract. Silver nanosuspension of 500 ppm was obtained using water as a solvent. Nanoparticle formation was observed using UV-vis spectroscopy in the range of 300-700 nm (Evolution 220 UV-Visible spectrophotometer).
After preparation, analysis of the influence of selected parameters on the physicochemical properties of obtained metallic nanosuspensions were conducted. Afterwards, stability analysis of obtained metallic nanosuspensions depending on the time, temperature and amount of infusion were tested. Material analysis was performed by HPLC and the antioxidant capacity of coffee samples was determined using Folina-Ciocalteu (F-C) and DPPH reagents.

Results and Discussion

The results of caffeine content measured by HPLC, antioxidant capacity by DPPH reagent and F-C reagent are shown in TABLE 1. Instant coffee and green coffee contain the highest amount of caffeine. This may be the result of the coffee preparation process. Contrarily to other coffee samples green coffee is not roasted. The highest antioxidant properties has instant coffee followed by Tchibo and Green coffee. Tchibo coffee was found the most efficient in SNPs preparation. In the time-dependent graph (FIG. 1), it can be seen that nanoparticles' synthesis proceeds the most effectively after 90 and 120 min. It can be observed that nanoparticles are relatively stable over time.

TABLE 1. Content of caffeine and antioxidant capacity evaluated by DPPH F-C reagents, respectively in different coffees used as reducing and stabilizing agents in SNPs synthesis.

Coffee	Caffeine content [mg/l]	% inhibition	Gallic acid [mg/ml]
Puro de altura coffee	2.89±0.03	8.60±0.01	0.29±0.02
Green coffee	5.37±0.01	11.65±0.09	0.32±0.02
Tchibo coffee	1.95±0.13	12.29±0.09	0.29±0.05
Instant coffee	7.18±0.05	40.75±0.06	0.46±0.05
Coffee from Nicaragua	3.52±0.12	2.81±0.07	0.21±0.02
Coffee from Mexico	2.95±0.02	5.06±0.03	0.18±0.03
Lavazza coffee	3.51± 0.02	8.33±0.03	0.24±0.04

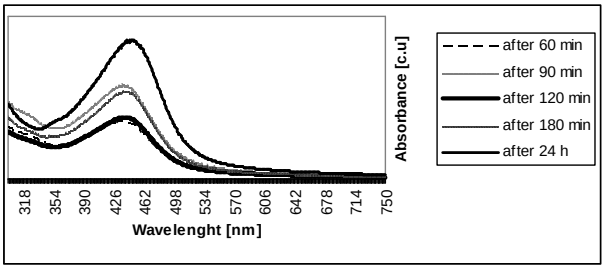

FIG. 1. UV-vis spectra of silver suspension from Tchibo coffee-water at room temperature depending on synthesis time.

Conclusions

Based on the study it can be concluded that different types of coffee have different caffeine content and antioxidant properties. The highest caffeine content and antioxidant properties has instant coffee, while the lowest caffeine content has Tchibo coffee. Extract from Tchibo coffee was found the most effective stabilizing-reducing agent in preparation of stable SNPs suspensions. There is not a clear relationship between the formation of SNPs and the antioxidant capacity of coffee.

Acknowledgments

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References

[1] Bhushan B.: Handbook of Nanotechnology, Springer 2004.
[2] Świderski F., Waszkiewicz-Robak B.: Nanotechnologia - teraźniejszość i przyszłość, Postępy techniki przetwórstwa spożywczego. 2006;1:55-57.
[3] Landage S.M., Wasif A.I., Dhuppe P.: Synthesis of nanosilver using chemical reduction methods, International Journal of Advanced Research in Engineering and Applied Sciences, Vol. 3; 2014, ISSN: 2278-6252, No. 5:1-9.
[4] Tien D.C., Liao C.Y., Huang J.C., et al.: Novel technique for preparing a nano-silver water suspension by the arc-discharge method. Reviews on Advanced Materials Science. 2008;18:750–756.
[5] Mroczek-Sosnowska N., Jaworki S., Siennicka A., Gondek A., Unikalne właściwości nanocząstek srebra, Polskie drobiarstwo, 2013;02:6-8.
[6] Alarcon E.I., et al.: The biocompatibility and antibacterial properties of collagen stabilized, photochemically prepared silver nanoparticles. Biomaterials 2012;33(19):4947-4956.

EFFECT OF SURFACE TITANUM MODIFICATION ON INTERACTION WITH GRAPHENE OXIDE COATINGS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 70]

Introduction

Titanium from many years is used in biomaterials engineering as a material with high biocompatibility. For years, their surface have been also modified to improve biocompatibility, bioactivity, chemical resistance, or gives special properties such as biomimetic, and change surface roughness or surface energy [1,2]. Among the various modifications of the titanium surface also coatings based on carbon nanomaterials are used [3]. One potential use of such systems is the electrodes for brain stimulation in the treatment of central nervous system diseases. In the design of electrodes for brain stimulation, it is important that the coatings adhere well to the titanium surface. The main purpose of the work was to modify the titanium surface with argon ion beam, pyrolytic coatings and chemical digestion to evaluate which of the proposed modifications have the best effect on the adhesion of the graphene oxide coatings.

Materials and Methods

The titanium (Gr2) plates in 0.5 mm thickness were used in this investigation. To modification of Ti surface three different methods were used namely: etching in 5%HF, chemical vapour deposition method (CVD) to synthesis pyrolytic layers and plasma enhanced chemical vapour deposition method (PECVD) for treatment of Ti surface of Ar ion beam. All samples were investigated using different methods like confocal microscopy for roughness measurement, goniometer for analysing of surface wettability and SEM for morphology and microstructure investigation. Then, on the treated Ti samples the graphene oxide (GO) coatings using electrophoretic deposition method (EDP) were prepared. The impact of titanium substrate modification on the adhesion of the GO coatings was determined by a scratch test using Micro-Combi-Test (MCT), CSM Co.

Results and Discussion

The analysis of SEM microphotographs of the treated Ti samples indicates that the morphology and microstructure were different and strongly depends on modification method (FIG. 1).

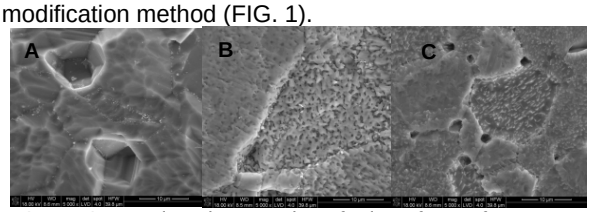

FIG. 1. SEM microphotographs of Ti surface after treatment using 5%HF (A), pyrolytic carbon (B), Ar ion beam (C).

Microstructural differences have also been confirmed by confocal microscopy (FIG. 2). The roughness value determined by this device confirms that the higher roughness is observed for the sample after HF etching (FIG. 2A). 5%HF is a agent which strongly interact with Ti surface changing significantly their morphology and topography. Pyrolytic carbon and Ar ions are not as aggressive as HF and the Ti surface roughness modified these agents is much lower.

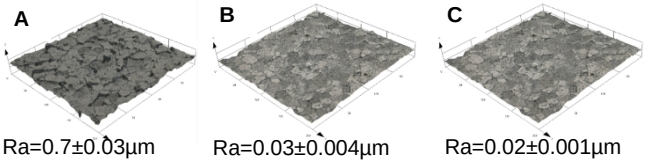

FIG. 2. 3D images of Ti surface after treatment using 5%HF (A), pyrolytic carbon (B), Ar ion beam (C), and values of roughness (Ra).

Treated methods of Ti surface have influence on surface wettability. The highest contact value was observe for Ti after modification using pyrolytic carbon in turn the lowest contact value which was observed for Ti after treatment in Ar ions (TABLE 1).

TABLE 1. Water contact angle for treated Ti samples.

Samples	Water contact angle [°]
Ti after HF	84.7±5.63
Ti with pyrolytic carbon	95.5±2.59
Ti after Ar ions beam	73.6±2.27

Verification of the modification methods of the Ti plates was done on Ti samples on which GO coatings were deposited using EPD method. All three samples with GO coatings was investigated using scratch test and the force value at which the first abrasion was observed and the force at which the GO coatings was completely destroyed were analysed (TABLE 2).

TABLE 2. Scratch test results.

Samples	Force initiating the destruction [N]	The force that destroys the coating [N]
Ti after HF	0.26	2.5
Ti with pyrolytic carbon	0.26	5.0
Ti after Ar ions beam	0.27	4.5

These result shows that not high roughness have the significant impact on adhesion of GO coatings to Ti surface but probably wettability and chemical affinity of the Ti surface to coatings is decisive. Pyrolytic carbon because it is a carbon material similar to the graphene oxide coatings are most likely to interact with each other which results is growing of destructive force. In the case of Ti after Ar ions beams modification, probably lower wettability of this surface in comparison with other materials have significant impact on increasing of destructive force of GO coating. The GO is hydrophilic materials which can interact stronger with surface characterised by higher wettability.

Conclusions

These results shows that Ti surface modification using different technique are successful in changing surface topography and wettability and increasing interaction between Ti and GO coatings deposited on its surface using EPD technique.

Acknowledgments

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References

[1] B. Fouziya, A.M. Uthappa *et al.* Journal of Advanced Clinical & Research Insights (2016), 3, 215–219
hnol. 61 (2001) 1189-1224.
[2] A. Ścisłowska-Czarnecka, E. Menaszek, B. Szaraniec et al. Tissue & Cell (2012), 44: 391–400.
[3] A. Fraczek-Szczypta et al. Appl. Phys. A. 123 (2017) 1-10.

CNTs ALTER THE BIOCOMPATIBILITY OF PAN-DERIVED CNFs

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[ENGINEERING OF BIOMATERIALS 143 (2017) 71]

Introduction

Carbon nanofibers are interesting materials that may find numerous applications in the field of advanced materials engineering. Among these, the most interesting are the ones that exploit their very high aspect ratio, good electrical and thermal conductivity as well biocompatibility [1-4]. Thus, many studies are devoted to applying the CNFs in filtering applications and as scaffolds for tissue engineering of tissues that are hard to regenerate: bone, neural, muscular and chondral.

In order to meet specific requirements for target application (e.g. surface chemistry, structural arrangement, mechanical and electrical properties), physicochemical modification needs to be performed [5-7]. This can be done either by modifying the CNFs precursors or by altering the already carbonized fibers. The former method is more interesting as in this way the modification is more efficient and permanent and is cheaper – no additional fabrication steps need to be performed.

CNTs are interesting candidates for matrix additives due to their biocompatibility, good mechanical properties [8,9] and reported ability to stimulate regeneration of various tissues [10]. Furthermore, by using different chemical modifications of CNTs, different interactions between the CNFs precursor and the additive are expected, yielding materials of varying qualities.

In this study, two types of CNTs were used as matrix additives of the CNFs precursors. The aim was to test the hypothesis whether presence of CNTs in the CNFs is able to alter the material's biocompatibility.

Materials and Methods

Polyacrylonitrile (11% wt.) was dissolved in DMF and to the obtained solution the CNTs were added (0.25% wt.). The obtained solutions were electrospun and the resulting mats were subjected to two-step thermal treatment: oxidation and carbonization. The resulting materials were subjected to physicochemical analysis. Finally, biocompatibility using NHOst cells was evaluated.

Results and Discussion

By using two types of CNTs, varying in the type and amount of functional groups, two types of carbon nanofibers, different from each other, as well as from the unmodified CNFs were obtained. The materials had different fibers' dimensions, surface chemistry and structural arrangement, with higher level of structural arrangement observed of the CNFs modified with highly oxidized CNTs. Presence of CNTs favoured cells' adhesion and directional growth. Again, the more oxidized CNTs were found to yield materials that are more biocompatible. Thus, the CNTs are summarized to be effective modificators of CNFs fibers and can be used to improve their biological properties.

Conclusions

The CNTs are summarized to be effective modificators of CNFs and can be used to improve their biological properties. Osteoblasts are found to favour CNFs modified with highly oxidized CNTs, having higher level of structural arrangement

Acknowledgments

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References

[1] N. Saito, K. Aoki *et al.,* Chem Soc Rev, 40 (2011) 3824-3834.
[2] A. Fraczek-Szczypta, Mat Sci Eng C, 34 (2014) 35-49.
[3] L. Zhang, A. Aboagye *et al.,* J Mater Sci, 49 (2014) 463-480.
[4] S.K. Nataraj, K.S. Yang, T.M. Aminabhavi, Prog Polym Sci, 37 (2012) 487-513.
[5] H.-H. Cho, B.A. Smith, J.D. Wnuk, D.H. Fairbrother, W.P. Ball, Environ Sci Technol, 42 (2008) 2899-2905.
[6] Y. Bai, Z.-H. Huang, F. Kang, Colloid Surface A, 443 (2014) 66-71.
[7] P. Thevenot, W. Hu, L. Tang, Curr Top Med Chem, 8 (2008) 270-280.
[8] T. Maitra, S. Sharma *et al.,* Carbon, 50 (2012) 1753-1761.
[9] Y. Liu, H.G. Chae, S. Kumar, Carbon, 49 (2011) 4466-4476.
[10] J. Beuvelot, C. Bergeret *et al.,* Acta Biomater, 6 (2010) 4110-4117.

MODIFICATION OF MICRO-SPHERES' MICROSTRUCTURE FOR APPLICATION AS CELL CARRIERS IN MODULAR TISSUE ENGINEERING

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[ENGINEERING OF BIOMATERIALS 143 (2017) 72]

Introduction

Microspheres (MS) made of resorbable polymer have been proposed as a convenient cell growth support. They may be assembled to form cell constructs or suspended in hydrogels allowing injection into injury location. High relative surface area of MS provides more efficient cell culture environment than traditional 2D culture on flat substrates (multiwall plates, Petri dishes). In addition, MS structure, topography and surface chemistry can be modified to promote cell adhesion and proliferation [1].
The aim of this study was to modify MS properties by varying manufacturing conditions of oil-in-water emulsification to better control structural and microstructural properties of MS and their biological performance.

Materials and Methods

MS1 were prepared by oil-in-water emulsification method by pouring dissolved in dichloromethane (20% wt/vol) poly(L-lactide-*co*-glycolide) (PLGA 85:15, M_n = 100 kDa, M_w = 210 kDa) oil phase into water phase supplemented with 1.5% PVA. MS2 were produced in the same manner but water phase was additionally supplemented with 0.5% NaCl. For MS3 manufacturing oil phase was modified with addition of 20% PEG (M_n = 400 Da). After 24 h MS were vacuum filtered, rinsed with distilled water, dried at 37°C, sieved and fraction >100 µm was collected. MS were analysed with optical microscopes (Axiovert 40, Zeiss and VHX-900F, Keyence), scanning electron microscope (Nano Nova SEM 200) and DSC (DSC1 from Mettler Toledo). DSC measurements were performed in the temperature range of -90÷200°C at heating rate 10°C/min in nitrogen atmosphere, sample mass was ca. 6 mg. MG-63 osteoblast-like cells were cultured in TCPS 48-well Nunclon plates containing 200 µl 10% MS suspension in 70% ethanol for 1, 3 and 7 days at 37°C under 5% CO_2. Cell viability was assessed by Alamar Blue assay (Sigma Aldrich), live/dead staining (calcein AM/propidium iodide, Sigma-Aldrich) with fluorescence and optical microscopy (Axiovert, Zeiss, Keyence VHX-900F).

Results and Discussion

Modification of water phase (MS2) and oil phase (MS3) resulted in differences in appearance, transparency and microstructure of the MS. Addition of NaCl to water phase caused high transparency and low porosity of MS2 (FIG. 1A). When oil phase was modified with PEG400 opposite effect was observed: MS3 were opaque and porous. Microstructure of MS differed between samples and depended on the used modification approaches but in general the surface of MS was smooth and small pores (few µm in diameter) were found. DSC results (FIG. 2) showed that MS differed in crystallinity: MS1 had melting enthalpy ΔH_m = -2.47 J/g, MS2 ΔH_m = -0.92 J/g while MS3 ΔH_m = -4.82 J/g.
Thus modification of water phase with NaCl, which increased its ionic strength, resulted in more amorphous PLGA forming MS. Addition of PEG to PLGA solution (modification of oil phase) resulted in increase in crystallinity, probably due to the fact that PLGA and PEG do not form a physical mixture but undergo phase separation as shown in our previous study [2]. The findings regarding crystallinity are important from the point of view of using MS in cell culture, because it is known that crystallinity influences degradation kinetics of PLGA.

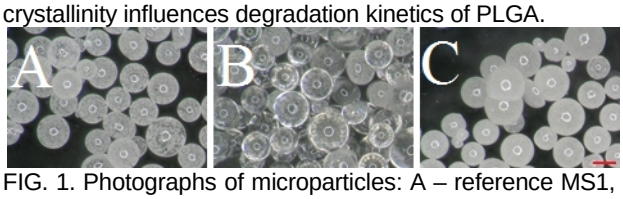

FIG. 1. Photographs of microparticles: A – reference MS1, B – MS2 – water phase modified with NaCl, C – MS3 – oil phase modified with PEG400. Scale bar 100 µm.

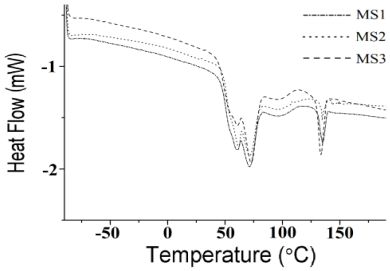

FIG. 2. DSC results of MS: reference MS1, MS2 – water phase modified with NaCl, MS3 – oil phase modified with PEG400.

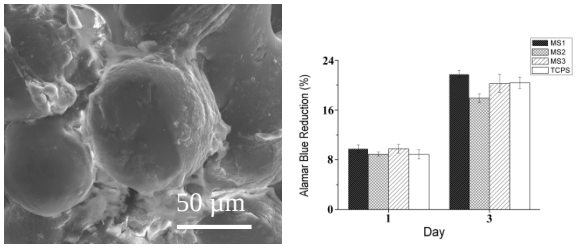

FIG. 3. SEM microphotograph of MG-63 cells on MS3 after 7 days of culture and Alamar Blue results.

In vitro tests showed good cytocompatibility of all MS. After 7 days cells grew on majority of microspheres and created cell-MS agglomerates (FIG. 3). Cell adhesion and proliferation on days 1 and 3 were similar to reference material (TCPS).

Conclusions

Method of emulsification is effective in manufacturing PLGA microparticles in diameter of >100 µm, of controlled crystallinity and morphology and allows for easy modification of these parameters by addition of NaCl and PEG, to water and oil phases, respectively. *In vitro* tests showed that MS support cell growth and formation of MPs-cell agglomerates which indicated their good cytocompatibility.

Acknowledgements

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References

[1] Hossain KMZ., Patel U., Ahmed I.: Development of microspheres for biomedical applications: a review. Prog Biomater 4:1-19. 2015.
[2] Krok M., Pamuła E.: Poly(L-lactide-*co*-glycolide) microporous membranes for medical applications produced with the use of polyethylene glycol as a pore former. J Appl Polym Sci 125:187-199. 2012.

POLYMERIC ANTICOAGULANTS BASED ON POLY(2-(ACRYL-AMIDO)-2-METHYLPROPANE-SULFONIC ACID) BLOCK POLYMERS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 73]

Introduction

Maintaining equilibrium between two opposite processes, i.e. blood coagulation and fibrinolysis, is essential for proper functioning of the body. Any deviation from this state is potentially life-threatening. On one hand, insufficient blood coagulation may result in haemorrhage and dangerous blood loss, while on the other hand excessive blood coagulation may be the reason of clot formation within the blood vessels leading to more or less blocked blood flow and severe pathologies such as ischemic stroke or infarct of an organ.

The need of lowering excessive blood coagulation resulted in the advent of an important class of drugs – anticoagulants. Unfortunately, many of anticoagulants currently applied in clinics do not possess an antidote, e.g. low-molecular weight heparins (LMWHs) or fondaparinux.

Previously, we have obtained a heparin binding copolymer (HBC) which inhibits heparin [1]. Conversely, in this study we have synthesized and investigated di- and triblock polymers which show anticoagulative properties and therefore are potential anticoagulants. Importantly these polymers form polyelectrolyte complexes (PECs) with HBC, which therefore may constitute an antidote for these polymers.

Materials

4-Cyanopentanoic acid dithiobenzoate (CPD) was synthesized according to the method reported by McCormick and coworkers[26]. *α*,*ω*-Bis-hydroxy poly(ethylene glycol) (HO-PEG-OH, number-average molecular weight M_n=9.40×10^3, degree of polymerization DP=227, molecular weight distribution M_w/M_n=1.06, Aldrich), 2-(methacryloyloxy)ethyl phosphorylcholine (MPC, 96%, NOF Corp.), 4,4'-azobis(4-cyanopentanoic acid) (V-501, 98%, Wako), 2-(acrylamido)-2-methylpropanesulfonic acid (AMPS, 95%, Wako), 4-hydroxy-2,2,6,6-tetramethylpiperidinyl-1-oxy (HTEMPO, free radical, 98%, Aldrich), sodium hydrogen sulfite ($NaHSO_3$, Fluka, solution for synthesis, 38–40% in water), potassium persulfate ($K_2S_2O_8$, Aldrich, 99.99%), dimethylsulfoxide (DMSO, HPLC grade, POCH Gliwice), Griess reagent (1% w/v sulfanilic acid/0.1% w/v *N*-(1-naphtyl) ethylenediamine-dihydrochloride in 2.5% v/v H_3PO_4, Sigma), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), trisodium citrate (≥99%), calcium sulfate (≥99.99%) (Sigma-Aldrich, Germany), activated partial thromboplastin time (aPTT) and prothrombin time (PT) reagents (Bio-Ksel, Poland), anti-factor Xa assay kits (Sekisui Diagnostics, USA), pentobarbital (Biovet, Poland), collagen (Chrono-log, USA), diagnostic kit for determination of calcium concentration (Cormay, Poland) were used as received. Water was purified using a Millipore Milli-Q system.

Methods

The block copolymers used in the studies were synthesized using reversible addition-fragmentation chain-transfer polymerization (RAFT).

Results and Discussion

Block copolymers containing PAMPS as the anionic block and PEG or poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) as the neutral or zwitterionic block, respectively, were synthesized with various block length. It was found that the copolymers increased activated partial thromboplastin time (aPTT) (FIG. 1), prothrombin time and showed significant anti-fXa activity.

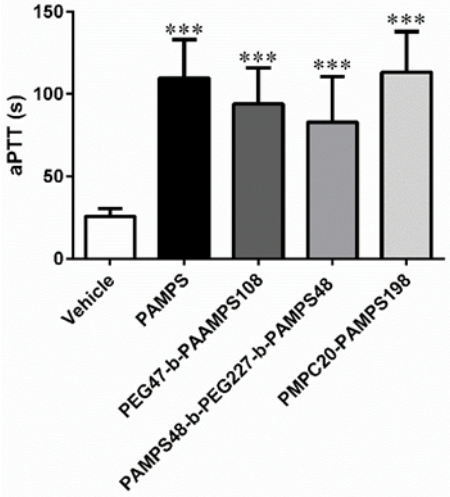

FIG. 1. Effects of PAMPS-based polymers on activated partial thromboplastin time (aPTT) in rats. ***p<0.01 vs. vehicle, unpaired Student's t-test. Results are shown as mean ± SD, n = 8-10.

Importantly, the polymers inhibited also platelet aggregation *in vitro*. However, in *in vivo* experiment the polymers inhibited, did not change or increased platelet aggregation. For PEG-PAMPS copolymers there was no change in the cardiorespiratory parameters, while for PAMPS homopolymer and PMPC-PAMPS copolymer with long PAMPS block a short term cardiac arrest and a significant decrease in the respiratory rate (RR) was found. All polymers significantly increased WBC in rats 30 minutes after intravenous administration. All the studied polymers showed anti-inflammatory properties.

Conclusions

The conclusions have to be based on the facts in evidence and should be limited to minimal speculation about the significance of the work.

Acknowledgments

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References

[1] B. Kalaska, K. Kaminski, *et al.*, Transl. Res. 177 (2016) 98–112S.

AROMATIC PEPTIDES AS COMPONENTS OF POTENTIAL SCAFFOLDS FOR REGENERATIVE MEDICINE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 74]

Introduction

Over the past few years, applications of the peptides were continued and extended in regenerative medicine. For instance, Thomas P. J. Knowles and co-workers have reported that in the naturally occurred β-amyloid deposits complex proteins, have been identified common motif composed of phenylalanine residues (FF) [1]. Additionally, Ehud Gazit proved that its structural feature upholding β-sheet structures is the relatively weak interactions between aromatic rings [2]. On the basis of the studies, a hypothesis was put forward that short-aromatic (hydrophobic) peptides could be used in medicine [3]. The aim of this study was to characterize biological effect of nonamer and hexamer peptides in cell culture and check whether the peptides could be used as components of scaffolds for regenerative medicine.

Materials and Methods

Materials

Six types of peptides with different composition (TABLE 1) were designed and synthesized in Institute of Organic Chemistry, Lodz University of Technology.

TABLE 1. Composition of peptides used in research.

3-letters structure representation	Symbol
H-PhePhePhePhePhePhe-OH	$(FFF)_2$
H-TrpTrpTrpTrpTrpTrp-OH	$(WWW)_2$
H-TrpTrpCysTrpTrpCys-OH	$(WWC)_2$
H-TrpTrpCysTrpTrpCysTrpTrpCys-OH	$(WWC)_3$
H-TryTyrCysTryTyrCys-OH	$(YYC)_2$
H-TryTyrCysTryTyrCysTryTyrCys-OH	$(YYC)_3$

Preparation of peptide layers

In the first step, 100 mg of peptides were dissolved in 100 ml of 70% ethanol. This Stock solution (0.1%) was diluted into two other concentrations (which were respectively 0.05% and 0.025%). In the second step, 300 µl of each solution was added into independent wells of the 96-well culture-plates. After that, the solvent was evaporated, resulting in the formation of peptide layers. All this procedure was conducted under sterile conditions.

Cell study

L929 mice fibroblasts (ATTC, USA) were cultured in Dulbecco's modified Eagle's medium DMEM (ATTC, USA) supplemented with 10% fetal bovine serum (ATTC, USA). The cells were cultured in optimal conditions at 37°C, 5% CO_2, and 95% humidity. After passage 3rd, cells were seeded on layers of peptides at a density of 5 x 10^3 cells per well (200 µl/well) and kept under culture conditions. The biocompatibility of the peptides was analyzed after 3 and 7 days of the culture with the use of PrestoBlue™ assay (Invitrogen, USA). The test was used to determine the amount of intracellular redox reaction of resazurin into fluorescent resorufin which corresponded with cells viability. In compliance with the manufacturer's protocols, 20 µl of the PrestoBlue reagent was added per well and plates were returned to an incubator for 1 h. The fluorescence was read at an excitation/emission wavelength of 560/590 nm on the microplate reader POLARstar Omega (BMG Labtech, Germany). Cells morphology was controlled using an optical microscope. All results were obtained by performing three independent repetitions of each measurement. All data were given as mean ± standard error of mean (SEM).

Results and Discussion

In this work, viability test was chosen to determine the biocompatibility of studied materials. The PrestoBlue assay confirmed the positive influence of studied peptides on cells viability. Obtained data are given in FIG. 1. In FIG. 2, the morphology of cells cultured in contact with the most effective peptides is shown.

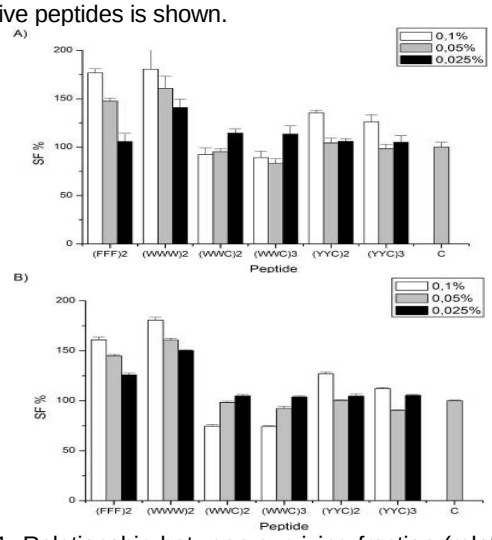

FIG. 1. Relationship between surviving fraction (relative to control) and the concentration of each peptide contacted with L929 fibroblasts for a) 3 days, and b) 7 days.

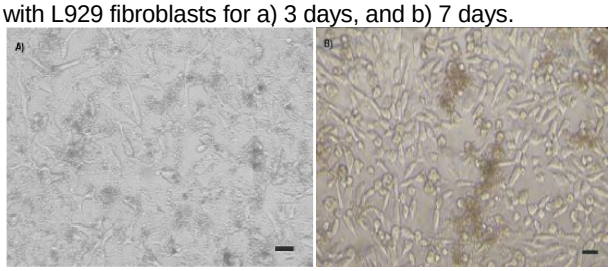

FIG. 2. Morphology of L929 cells cultured 3 days with a) $(FFF)_2$ and b) $(WWW)_2$. Scale bar 100 µm.

Conclusions

Based on the data gathered from viability test and obtained cells' morphology pictures, it is proven that the synthesized materials are not cytotoxic and do not negatively influence the growth of cells. Moreover, homohexamer peptides ($(FFF)_2$, $(WWW)_2$) are very effective in accelerating the proliferation and stimulating the activity of L929 fibroblasts.

Acknowledgments

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References

[1] T. P. J. Knowles, M. J. Buehler, Nat. Nanotechnol. 6 (2011) 469-479.
[2] E. Gazit, Nanobiotechnol. 1(3) (2005) 286-288.
[3] S. Marchesan, A. V. Vargiu, K. E. Styan, Molecules 20(11) (2015) 19775-19788.

BACTERICIDAL PROPERTIES OF Cu DOPED TiO_2 FILMS DEPOSITED ON MEDICAL STAINLESS STEEL

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[ENGINEERING OF BIOMATERIALS 143 (2017) 75]

Introduction

Due to its photocatalytic activity under the effect of UV light, titanium dioxide (TiO_2) has been found useful in a scope of health care and environmental protection applications [1,2]. Recently, a number of reports also appeared where the matrix made of a thin TiO_2 film hosts minority metal additions [3-5]. It is mainly made in order to intensify the photocatalysis, to extend recombination times and to shift the excitation threshold energy towards visible light. Apart from that, a small supplement of bactericidal activity exhibiting metal (such as Cu) may additionally enhance material's microbiocidal properties and substantially reduce bacterial colonization of the surface coated [3,4]. In the majority of these publications, TiO_2 coatings are deposited onto glass or quartz substrates, either with magnetron sputtering or with the sol-gel method. There are also relatively rare reports dealing with the synthesis of such coatings on a polymer or steel support [6], a use of which allows one to broaden the scope of applications with prosthetic devices, implants and other medical appliances. This is particularly important today, in the times of excessive overuse of antibiotics and strong mutagenic disinfection chemicals bringing new microbiological hazards. Under these circumstances, a search for novel alternative means of bacterial prevention and control is well under way [1-3].

Materials and Methods

Copper doped TiO_2 coatings were produced with the help of the radio frequency plasma enhanced chemical vapor deposition (RF PECVD) method. Medical grade SANDVIK BIOLINE 316LVM (ISO 5832-1) steel was used to prepare substrates. Chemical composition of the coatings was determined with X-ray photoelectron spectroscopy (XPS), while their surface topography was assessed with atomic force microscopy (AFM). In addition, their phase composition was studied with the low angle X-ray diffraction (XRD) technique and chemical bonding was investigated with both Fourier transform infrared (FTIR) and Raman spectroscopies. Mechanical properties of the films, such as adhesion force, hardness and modulus of elasticity were assessed with the nanoindentation technique. Finally, bacteriostatic and bactericidic properties of the coatings as well as their water wettability under the effect of UV light illumination were also investigated.

Results and Discussion

The content of Cu in the coatings amounted to 0.38, 1.42 and 3.39 atomic percent. Due to an application of Cu (II) (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate) as a copper precursor, fluorine is also incorporated into the material and since it is a light element, its incorporation proceeds substantially faster than that of copper. To illustrate this, one has to note that a coating containing 3.39 atomic percent of copper also comprises 9.43 atomic percent of fluorine. In other words, a presence of copper in this case is inseparably connected with an access of fluorine.

In the AFM image of the undoped coatings, one can observe a presence of globular forms of small dimensions as well as their agglomerates of sizes attaining 120 nm. An addition of the dopand brings about surface homogenization and small globules (grains) are only observed. Results of Raman studies, confirmed with those of XRD investigations, reveal a predominantly amorphous character of the coatings. The only maxima present in the Raman spectrum are broad and of low intensity which suggests sole low distance interactions. In any case, the highest intensity of the peaks is recorded for the coatings doped with small amounts of copper. At the highest Cu concentration, most of these maxima disappear.

FTIR studies of the coatings confirmed a presence of chemical moieties characteristic for TiO_2. Hardness of a plain titanium dioxide coating amounts to 8.9 GPa and the smallest addition of the dopand results in its increase to 11.2 GPa. Farther doping, however, brings about reduction of this parameter. Similar are the changes of Young modulus.

In order to assess bactericidity of the coatings, a number of *E. coli* strain DH5 α bacteria adhered to their surface was counted. On the surface of plain titanium dioxide coating, 19.7% cells were computed relative to the surface of 316LVM steel used as the control. A small increase of the number of bacteria was recorded in the case of the smallest addition of copper (0.38%) but the larger additions reduced that number to approximately 10%. This is an evidence of the fact that doping a TiO_2 coating with copper at its concentrations higher than 1% enhances the bacteriostatic effect of this material.

Conclusions

With a help of the RF PECVD technique, it is possible to produce copper doped TiO_2 coatings, with an addition of the dopand increasing the coating homogeneity. All the coatings are characterized by amorphous structure. An addition of small amounts of copper brings about an increase of the coating hardness and its Young modulus. Finally, a substantial increase of the coating bactericidity is observed for the coating containing 3.39 atomic % of copper.

References

[1] V. Binas, D. Venieri et al D. Kotzias, G. Kiriakidis, J. Materiomics 3 (2017) 3-16
[2] S. Sontakke, J. Modak et al., Chem. Eng. J. 165 (2010) 225-233
[3] V.I. Syngouna, C.V. Chrysikopoulos, J. Colloid Interface Sci. 497 (2017) 117-125
[4] A. Markowska-Szczupak, K. Ulfig et al., , Catal. Today 169 (2011) 249-257
[5] H. Wang, R. Zhang et al., Ceram. Int. 41 (2015) 11844-11851
[6] K. Hashimoto, H. Irie, A. Fujishima, Jpn. J. Appl. Phys. 44 (2005) 8269–8285.

VATERITE $CaCO_3$-COATED POLYMERIC FIBROUS SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 76]

Introduction

The designing of new hybrid biomedical materials based on combination of organic and inorganic components is promising candidate for application in regenerative medicine. Recent development in materials science and bioengineering offers opportunities to design smart scaffolds, which are capable to imitate living tissues and stimulate formation new native tissues [2]. Functionalization of polymeric scaffolds is a promising approach to achieve biomimetic and bioactive properties. In this research, we have developed novel composite scaffolds based on polymeric polycaprolactone fibers coated with porous calcium carbonate structures (PCL/$CaCO_3$) for tissue engineering and have shown their drug delivery and release in rats.

Materials and Methods

To obtain PCL/$CaCO_3$ scaffolds, the electrospun PCL matrix was produced in the first step by using an electrospinning technique. The mineralization of the PCL matrix was carried out by using method introduced previously [2]. In vivo experiments, white nonlinear male rats were used. The scaffolds were subcutaneously implanted in the interscapular area of rats. After 21 days of implantation, the scaffolds were explanted. Initial and explanted scaffolds were examined by histological studies, scanning electron microscopy and X-ray diffraction.

Results and Discussion

The schematics and real imaging of obtained PCL/$CaCO_3$ scaffolds can be seen in FIG. 1. The route of PCL electrospun scaffold mineralization is shown by the scheme. As it can be seen, microfibrous matrix of the PCL/$CaCO_3$ scaffold is covered with the porous particle-like $CaCO_3$. These porous spherical $CaCO_3$ microparticles called vaterite proved their potential for biomedical applications including bone regeneration and drug delivery [3,4]. Therefore, modification of PCL matrix with vaterite coatings could significantly improve osteoconductive and bioactive properties of polymeric scaffold.

To study biocompatible and implantable properties of PCL/$CaCO_3$ scaffolds, in vivo tests were carried out with rats. The results of histological study demonstrated capability of PCL/$CaCO_3$ scaffold to be colonized by fibroblastic elements and vascularized without promoting inflammatory response in surrounding tissues in the course of subcutaneous implantation tests in white rats that proved its biocompatibility.

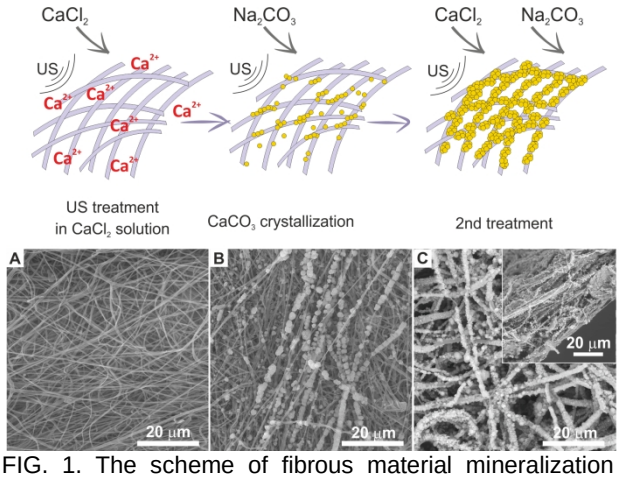

FIG. 1. The scheme of fibrous material mineralization under ultrasound treatment and corresponding SEM images of blank PCL fibrous material (A), PCL material on initial mineralization stage (B), and scaffold with uniform $CaCO_3$ coating after second treatment and cross-section of this scaffold (C) [2].

Conclusions

In this work, we have designed new PCL/$CaCO_3$ scaffolds, which were found to demonstrate a high degree of biocompatibility in vivo.

Acknowledgments

The ERA-NET Rus Plus program is acknowledged for support in the framework of the project “Fabrication and investigation of new hybrid scaffolds with the controlled porous hierarchy for bone tissue engineering” (Intelbiocomp). B.P. thanks Research Foundation Flanders (FWO), Belgium, for a postdoctoral fellowship.

References

[1] T. Dvir, et al., Nat. Nanotechnol. 6 (2011) 13–22.
[2] M.S. Savelyeva, et al., J. Biomed. Mater. Res. Part A. 105 (2017) 94–103.
[3] H. Maeda, et al., J. Mater. Sci. Mater. Med. 18 (2007) 2269–2273.
[4] B. V. Parakhonskiy, et al. ChemPhysChem. 15 (2014) 2817–2822.

BIOCERAMIC MICRO AND NANOPARTICLES AS FUNCTIONAL BIOLOGICAL MATERIALS

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[Engineering of Biomaterials 143 (2017) 77]

Introduction

Calcium carbonate is an important inorganic biomaterial thanks to its chemical stability, bioactivity, and biocompatibility. These properties have recently made it an interesting candidate for drug delivery systems [1]. Calcium carbonate exists in three anhydrous polymorphic modifications: vaterite, aragonite, and calcite. Under normal conditions, vaterite is an unstable phase [2,3], while calcite and aragonite are stable. The transition between these phases can be exploited as a payload release mechanism. Vaterite polycrystalline particles have further favorable properties like high porosity, large surface area, and negative zeta potential.

Materials and Methods

Spherical calcium carbonate microparticles (with a mean diameter of 3.0 ±0.3 μm) were synthesized via the protocol of Volodkin et al. [4] The same procedure of $CaCO_3$ particle synthesis was used for the formation of calcium carbonate microparticles with the mean diameter of 1.0 ±0.1 μm using the protocol described by Svenskaya et al. [5] briefly, but there is the only difference that stirring of the reaction mixture was carried out with ultrasound (US) with a frequency of 20 kHz and power density 1 W/cm^2 during 1 min. For the formation of calcium carbonate microparticles with the mean diameter of 0.5 ±0.2 μm the protocol developed by Parakhonskiy et al. was used [6].

Results and Discussion

In our work we present a novel technique for the synthesis and characterization of $CaCO_3$ containers. Porous polycrystalline particles were fabricated with controllable average sizes from 400 nm up to 10 microns. Fluorescent anticancer drug - photosensitizer was encapsulated to study payload release dynamics. Several levels of control on these release dynamics could be identified:

1) The immersion medium: capsules immersed in water, showed a delayed burst release of the dye, coinciding with the crystal phase transition from vaterite to calcite. In ethanol this phase transition was inhibited, consequently only a slow desorption of the encapsulated dye was found.

2) Surface modification: Covering microcontainers with additional layers of biocompatible polyelectrolyte increases the payload release time.

3) pH value: A change of the pH from neutral to acid conditions will instead lead to a destruction of the vaterite matrix leading to an immediate release.

Moreover, we report on studies of vaterite containers in cell culture assays, evaluating their cytotoxicity, their influence on cell viability, and the particles' uptake efficiency. The prove of principle to use such particles with encapsulated photosensitizer for photodynamic therapy were demonstrated.

Conclusions

The demonstrated flexible control released mechanisms, mild loading conditions and the perfect biocompatibility have proven the system's potential for future applications as drug delivery system, bioimplants and tissue engineering area.

Acknowledgments

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References

[1] Y.I. Svenskaya, A.M. Pavlov, D.A. Gorin, D.J. Gould, B.V. Parakhonskiy, G.B. Sukhorukov, *Colloids Surfaces B Biointerfaces* (2016), *146*, 171–179.
[2] Y. Svenskaya, B.V. Parakhonskiy, A. Haase, V. Atkin, E. Lukyanets, D.A. Gorin, R. Antolini, *Biophys. Chem.* (2013), *182*, 11–15.
[3] B.V. Parakhonskiy, C. Foss, E. Carletti, M. Fedel, A. Haase, A. Motta, C. Migliaresi, R. Antolini, *Biomater. Sci.* (2013), *1*, 1273.
[4] D.V. Volodkin, A.I. Petrov, M. Prevot, G.B. Sukhorukov, *Langmuir* (2004), *20*, 3398–3406.
[5] Y.I. Svenskaya, H. Fattah, A.M. Zakharevich, D.A. Gorin, G.B. Sukhorukov, B.V. Parakhonskiy, *Adv. Powder Technol.* (2016), *27*, 618–624.
[6] B.V. Parakhonskiy, A. Haase, R. Antolini, *Angew. Chemie Int. Ed.(*2012), *51*, 1195–1197.

COVERSION AND POLYMER-BASED COATINGS AS STRATEGIES TO INCREASE CORROSION RESISTANCE OF BIODEGRADABLE MAGNESIUM-CALCIUM ALLOYS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 78]

Introduction

Currently, magnesium and its alloys are considered to be one of the most promising metallic biomaterials. They are biocompatible, biodegradable, bioactive, have density similar to that of cortical bone and Young's modulus significantly lower than e.g. titanium-based materials [1,2]. Mg-based biomaterials with desired properties can be obtained by purification or alloying with Ca, Zn, Mn or other elements. Among those alloying elements, calcium is of particular interest due to its crucial role in natural bone and some evidence that co-release of Mg and Ca ions might be advantageous to the bone healing. Magnesium and its alloys are beneficial in many aspects, nevertheless one cannot forget about the need to strictly control their degradation behaviour since rapid corrosion in physiological environment can lead to serious cell and tissue damage [1-4].
The aim of this study was to combine two different approaches to slow down corrosion rate of binary MgCa alloy - appropriate surface treatment and polymer-based coatings. Conversion coatings, beside reducing the degradation rate, were used to increase the surface roughness of Mg alloy samples, and hence improve the adhesion of the polymer-based coating.

Materials and Methods

Magnesium-calcium alloys were produced in the light metal foundry (Institute of Non-Ferrous Metals in Gliwice, Light Metals Division). Conversion coatings were prepared on the alloys by an electrochemical treatment in the KOH+KF solution at 20°C, at a current density of 5 A/dm^2 within 5 minutes. Conversion interlayer was characterized by surface roughness (Hommel-Etamic W10 profilometer) and thickness measurements (Dualscop MP-20), as well as by scanning electron microscopy (SEM) and immersion test in Ringer's solution. Polymer-based coatings were used in the next step. MgCa samples were coated either with pure poly(ε-caprolactone) (PCL, Sigma-Aldrich, Mn = 80 000) or a composite system in which polymer matrix was modified with tricalcium phosphate (TCP, Sigma-Aldrich® ($Ca_3(PO_4)_2$ ≥96.0%)) and zinc oxide (ZnO, Avantor Performance Materials Poland S.A.). Tested samples were incubated in phosphate buffered saline (PBS, Sigma-Aldrich) and simulated body fluid (SBF) in 37°C. SEM with EDS, FTIR, pH measurements, release of Mg ions and release of H_2 were used to test the samples.

Results and Discussion

In order to protect magnesium alloy substrates from rapid degradation and enhance its biological performance two strategies were combined. In the first step, conversion coatings were created on the surface of the MgCa alloy samples. Next, biodegradable polymer-based coatings were applied, in which TCP was introduced to the PCL matrix to improve bioactivity, while ZnO was added to ensure antibacterial properties.
The coating produced in the KOH+KF solution uses potassium, fluorine, oxygen and magnesium. All these elements are beneficial to the human body and most importantly, they are not dangerous.

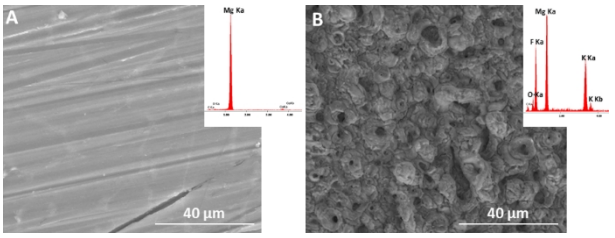

FIG. 1. SEM images with EDS analysis of (A) MgCa and (B) MgCa sample with conversion interlayer.

SEM analysis (FIG. 1) confirmed successful fabrication of conversion coatings on the surface of the MgCa samples (with EDS showing Mg, F, K and O). Surface treatment affected microstructure resulting in higher surface roughness. MgCa samples were further successfully coated with homogenous layer of polycaprolactone alone or composite system with TCP and ZnO particles that were uniformly distributed in the polymer matrix.
Hydrogen release studies showed significant reduce in the amount of H_2 released when the composite PLA/TCP/ZnO coating was used. Both chemical composition of the polymer coating and pretreatment of magnesium alloy (conversion coating) influenced morphology and composition of precipitates observed on the surface of the samples incubated in simulated biological fluids (PBS and SBF). Deposition of magnesium oxide on the surface of all the incubated samples and formation of calcium-phosphate or magnesium-phosphate precipitations was observed. It points to potential bioactivity of the conversion- and polymer-coated MgCa samples.

Conclusions

It was proven that combination of conversion coating with polymer-based coating reduces resorption rate of the MgCa binary alloy and improves their biologically relevant properties. The conversion coating increased the surface roughness of the Mg elements, thus increasing the adhesion of the polymer-based layer. Further studies, including bacterial activity and in vitro biocompatibility assays (with appropriate cell cultures) are needed.

Acknowledgments

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References

[1] N. Li, Y. Zheng. J. Mater. Sci. Technol. 29(6) (2013) 489-502.
[2] S.C. Cifuentes, R. Gavilan, M. Lieblich, et al. Acta Biomaterialia. 32 (2016) 348-357.
[3] A. Drynda, J. Seibt, T. Hassel, et al. J. Biomed. Mater. Res. A. 101(1) (2013) 33-43.
[4] M. Yazdimamaghani, M. Razavi, D. Vashaee, et al. Mater. Sci. Eng. C Mater. Bio. Appl. 49 (2015) 436-44.

INTEGRATION OF TRACE ELEMENTS INTO CALCIUM PHOSPHATE COATINGS ON TITANIUM AND THEIR CHARACTERIZATION *IN VITRO*

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[ENGINEERING OF BIOMATERIALS 143 (2017) 79]

Introduction

The aim of this study was to combine the trace elements copper and strontium with inorganic surface modification techniques already successful in bone applications. Copper ions released from bone implant coatings are known to enhance vascularization [1] while the incorporation of strontium is intended to stimulate bone forming and simultaneously suppress bone-resorbing cells in the implant vicinity. Calcium phosphate phases (CPP) were utilized as a carrier for the selected trace elements in order to i) provide osteoconductive surfaces and ii) to tune the immobilized amounts and release behavior of the trace elements.

Materials and Methods

Deposition of brushite onto c.p.Ti discs was performed by means of electrochemically assisted deposition (ECAD) from aqueous electrolytes containing $Ca(NO_3)_2$, $NH_4H_2PO_4$ with or without additional $Sr(NO_3)_2$. Cu integration was realized by three approaches: i) galvanostatically prior to the CPP deposition, ii) addition of $Cu(NO_3)_2$ to the electrolyte and iii) adsorption after the ECAD process.
The coatings were characterized by chemical analysis after dissolution in 0.1 M HNO_3. Ion release from coated surfaces was analyzed after incubation in simulated body fluid (SBF) with or without 10% fetal bovine serum (FBS). Human monocytes were isolated from buffy coats by $CD14^+$ labelling [2]. Their ability to differentiate into osteoclasts was studied for cultivation on coated samples differing in their trace element content as well as to cells cultured on tissue culture polystyrene (TCPS) for application of several combinations of trace elements in solution. Impact of the same trace element solutions onto osteogenesis was studied with human bone marrow stromal cells (hMSC).

Results and Discussion

If Cu and Sr ions were added during the ECAD process their deposited amount depended mainly on the concentration of the respective ions in the electrolyte. While addition of 0.5 mM Cu resulted in up to ~100 µg Cu/cm² a comparatively high Sr concentration (40 mM) was necessary to obtain a similarly high Sr deposition. While Cu adsorption was limited to ~50 µg/cm² for coatings composed of ~1000 µg/cm² brushite content, the galvanostatic Cu deposition could be increased up to several mg/cm². No Cu release was detected for incubation of all types of Cu containing coatings in serum-free SBF, while a permanent Sr release was observed in serum free SBF. In contrast, in presence of 10% serum considerable amounts of Cu (50% and 54%, respectively) were released if Cu was adsorbed or deposited as base layer within the first 24 h. Sole incorporation of Cu during the ECAD process (method ii) could reduce initial release to ~13%. However, co-deposition of Cu and Sr by the same method ii resulted in an accelerated release of Cu with 89% being detected within the first 24 h, Sr release on the other hand was nearly unaffected.
For single Sr incorporation the release behavior in presence of serum was comparable to serum free solutions for the first 24 h, but dropped to about one third of the respective amount in serum-free SBF irrespective of the initial Sr content. Continued steady release was monitored for up to 22 d.
In the first cell studies all coatings containing 100 µg/cm² Cu or more prevented the adhesion of monocytes as well as of hMSCs. The presence of Sr did not affect monocyte but clearly improved hMSC adhesion.
The impact of several combinations of Cu and Sr in solution was studied for both cell types. The presence of low concentrations of Cu (beginning from 25 µM) resulted in an increased proliferation of hMSC without osteogenic supplements that was even enhanced for application of both ions together. However, in presence of osteogenic supplements even the lowest concentration of Cu (25 µM), that was only slightly above the serum level, resulted in extreme reduction in cell number.
If monocytes were cultured in presence of Cu these ions provoked decreases in adherent cells for concentrations >50 µM while Sr had no impact on cell adherence. For both ions already at 25 µM a change in osteoclast cell fusion capability was observed (FIG. 1). The activity of tartrate resistant acid phosphatase was not affected by Sr for up to 1000 µM but increased by Cu even for the lowest concentration of 25 µM.

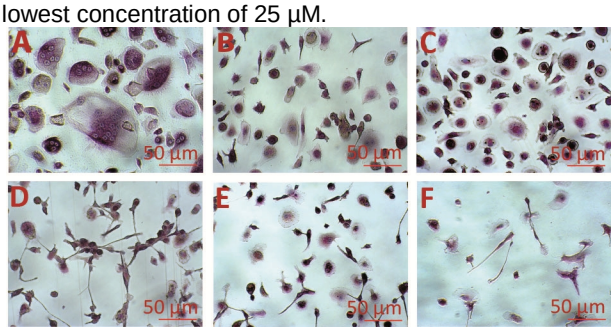

FIG. 1. TRAP staining of human monocytes after 8 d culture on TCPS with addition of A) no trace elements, B) 25 µM Sr, C) 1000 µM Sr, D) 25 µM Cu, E) 25 µM Cu & 25 µM Sr, F) 25 µM Cu & 1000 µM Sr.

It was shown that Cu and Sr can be co-deposited together with calcium phosphate onto metallic implant materials by ECAD. The deposited amount of these ions is tunable in a wide range. The combination of Cu and Sr seems to provoke a suppression of osteoclast differentiation while impact on hMSC depends on the presence of osteogenic supplements. Current investigations focus on fine-tuning of the deposited Cu amount to achieve optimal balance for osteogenic differentiation and osteoclast suppression.

Acknowledgements

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References

[1] Barralet J. *et al.*, Tissue Eng. A 15**:** 1601-1609. 2009
[2] Bernhardt A. *et al.,* Tissue Eng. C 21: 160-170, 2014

CARBON NANOTUBES, CARBON NANOFIBERS - *IN VITRO* STUDY

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[ENGINEERING OF BIOMATERIALS 143 (2017) 80]

Introduction

Fibrous carbon nanomaterials, i.e. carbon nanotubes (CNT) and carbon nanofibers (CNF) have found a number of applications in environmental protection and in medicine [1]. The reaction of cells to a material depends on its physical and chemical properties and has a decisive influence on the type of application in the field of medicine. CNT and CNF constitute also valuable initial materials for the manufacture of biosensors, electrodes and fibrous substrates in the form of 2D or 3D structures that meet the requirements of regenerative medicine. In each of these materials biocompatibility of degradation products is a key element.

The aim of the study was to investigate *in vitro* carbon nanomaterials in contact with cells. It is already well recognized that carbon nanotubes after an oxidative treatment are particular useful materials for medical purposes, whereas the as-received (non-functional) nanotubes may have a toxic impact on living systems [2]. By introducing certain quantities of materials into the cell culture, efforts were made to demonstrate differences in the behavior of cells in contact with CNT or CNF and to explain their essence based on the characteristics of individual nanomaterials.

Materials and Methods

Carbon nanotubes from Nanostructured & Amorphous Materials, USA and carbon nanofibers manufactured from PAN-Zoltek, Hungary nanofibers precursor made in the electrospinning process followed by carbonization at 1000^{o}C were investigated. Carbon nanomaterials were characterized using FTIR spectroscopy and SEM microscopy. Multiwalled carbon nanotubes (MWCNT), nanotubes functionalized in a mixture of acids (MWCNTf), carbon nanofibers (CNF) and oxidized nanofibers (CNFf) (after grinding process), were immersed in PBS (1 mg / 1 ml) and homogenized. The *in vitro* tests were performed in contact with RAW 264.7 macrophage and L929 fibroblasts lines. Biological evaluations of carbon nanomaterials were conducted using the following in vitro tests; viability cells (PrestoBlue), the cytotoxicity (Toxi Leight) and detection of active oxygen (DCFH-DA.

Results and Discussion

SEM studies indicate that both carbon groups, i.e. CNT and CNF, have significantly different geometry and particle sizes (FIG. 1). Nanomaterials, which are the subject of research, also differ in the structure and chemical state of the surface. CNT are materials with a precisely defined structure in which the graphene layers form a series of coaxial rolled sheets, while carbon nanofibers, formed from the electrospun PAN precursor, constitute a fine crystalline carbon phase performing characteristic turbostratic structure. The surface of CNTf, contains oxygen-containing chemical groups, e.g. hydroxyl and carboxyl groups, and due to presence of nitrogen from polymer precursor in carbon residue after annealing to 1000^{o}C, nitrogen containing functional groups. Biological studies have shown that the functionalized materials, both nanotubes and nanofibers, are characterized by improved biocompatibility compared to the initial materials. The highest cytotoxicity was found for carbon nanofibers that have not been pre-oxidized. On the other hand, all the materials tested induced low oxidative stress, significantly lower than that manifested by control (FIG. 2).

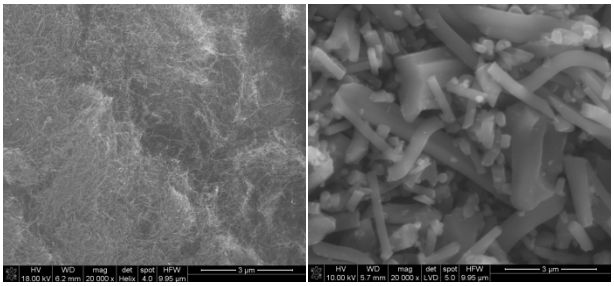

FIG. 1. SEM images of carbon nanotubes (MWCNT) and carbon nanofibers (ESCNF) after grinding process.

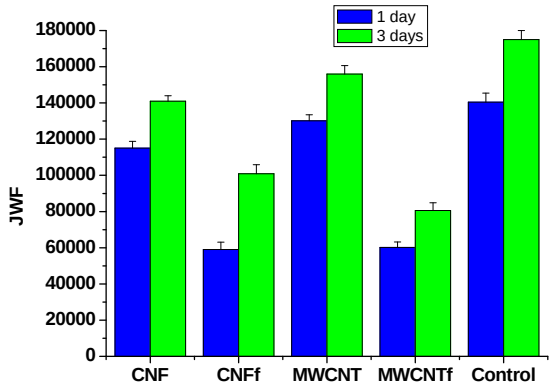

FIG. 2. The level of all reactive oxygen species produced by macrophages in contact with carbon nanomaterials.

Conclusions

Fibrous carbon nanomaterials are valuable materials for a number of medical applications. However, these materials require post-manufacture processing that changes the chemical nature of the surface and removes elements that can significantly reduce their biocompatibility.

A high relative cytotoxicity of the nanofiber degradation products formed during the carbonization of the PAN nanofibers precursors may be due to the presence of some chemical groups on the nanofibers surface that may be toxic to cells.

Acknowledgments

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References

[1] D.A. Stout, B. Basu, and T.J. Webster, Acta Biomaterialia 7, 3101 (2011).
[2] A.A. Shvedova, A. Pietroiusti, B. Fadeel, VE. Kagane, Toxicology and Applied Pharmacology 261, 121 (2012)

SUITABILITY OF GRADIENT COATINGS OF TiO_2/SiO_2 IN IMPROVING HEAMOCOMPATIBILITY OF AISI 316LVM BASE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 81]

Introduction

Many years of biotolerance studies AISI 316LVM allowed to establish certain qualitative and quantitative criteria ensuring the safety of its use and assemble it to the standards. This applies above all to the chemical and phase composition and mechanical properties. Suitable amounts of dopant elements provide paramagnetic structure and corrosion resistance in tissue environment. Nevertheless solutions don't solve existing problems concerning with steel implants attributed to the formation of physico-chemical properties of the surface layer. The main aim of the surface layer is to prevent adverse phenomenon generated by the implant in the blood environment [1-3]. The essence of the problem in the proposed study was to evaluate the coating of TiO_2/SiO_2 for improving heamocompatibility of AISI 316LVM used among others for blood-contacting implants.

Materials and Methods

The coating for steel substrate (after electrochemical polishing and chemical passivation) were coated by ALD method (SiO_2: precursors - $C_6H_{19}N_3Si$ and O_3, T = 340°C, L = 600 cycles; TiO_2: precursors - $TiCl_4$ and H_2O, T = 200°C, L = 50 cycles). The application of this type of surface treatment did not affect for the structure and mechanical properties of the base material. Surface topography was performed by atomic force microscope (AFM). The mechanical properties of the surface layer with TiO_2/SiO_2 layer determined on the basis of nanometer hardness (nH) and Young's modulus (Ym).
In turn, adhesion tests and other symptoms of mechanical damage of layer were performed by scratch test method used by open platform equipped with CSM Micro-Combi-Tester. Pitting corrosion resistance (DC-ET) was evaluated by recording of polarization curves potentiodynamic method using the AutoLab PGSTAT 302N in a three-electrode system. In addition, our research was using supplemented electrochemical impedance spectroscopy (EIS) was carried out. The measurement was performed using the same set of measurement as in the potentiodynamic tests equipped with a FRA2 module (Frequency Response Analyser). All the electrochemical tests were performed in artificial plasma solution (T = 37 ±1°C, pH = 6.8 ±0.2).

Results and Discussion

Studies carried out with the use of atomic force microscopy showed no significant differences in surface topography. The morphological characteristics of the SiO_2/TiO_2 layers showed a tendency to inherit the stereometric parameters of the steel base surface formed by treatments preceding its application. The surface roughness expressed by the Ra parameter for the base and the layer was in the range of 7 ±1 nm. The hardness of the layer was measured at a depth of 30 nm from its surface was H_{IT} = 11947 MPa and was higher than the hardness of the steel base - H_{IT} = 4478 MPa. In other hand, the value of the critical force determined in the Scratch –test caused total delamination was L_{c2} = 4.22 N. During the test, no cracks or chipping were observed. There was also no sound signal. This indicates a low binding energy between the layer and the base. In turn, the obtained polarization curves in potentiodynamic studies were the basis for determining the characteristic electrical properties describing the resistance to pitting corrosion – TABLE 1. The presented results unequivocally demonstrated that, the layer with Ti and Si causes increase corrosion potential, polarization resistance and transpassivation potential, which is a favorable phenomenon.

TABLE 1. Results of potentiodynamic test.

Sample	$E_{corr,}$ mV	E_{tr}, mV	Rp, kΩcm^2
AISI 316LVM	-288	+1203	2820
AISI 316LVM + TiO_2/SiO_2	-148	+1622	28524

Increase of corrosion resistance by the presence of the layer was also confirmed in EIS studies. Impedance spectra for the TiO_2/SiO_2 layer were interpreted by comparing them to equivalent circuit, which indicates the appearance of two sublayers: internal compacted and external porous (R_s – artificial plasma resistance, R_{pore} – pore layer resistance, CPE_{pore} – the capacity of double layer (porous, surface), R_{ct} and CPE_{dl} – resistance and capacity of TiO_2/SiO_2 layer) [4] – TABLE 2.

TABLE 2. Results of EIS.

Sample	R_s, Ωcm^2	R_{pore}, Ωcm^2	CPE_{pore}		R_{ct}, kΩcm^2	CPE_{dl}	
			Y_0, Ω^{-1} cm^{-2}s^{-n}	n		Y_0, Ω^{-1} cm^{-2}s^{-n}	n
AISI 316LVM	17	-	-	-	2517	0.1887E-4	0.87
AISI 316LVM + TiO_2/SiO_2	16	19	0.6597E-4	0.85	32105	0.6685E-4	0.87

Conclusions

The passive layer made on AISI 316 LVM base during surface pretreatment (mechanical and electrochemical) and deposition the gradient TiO_2/SiO_2 layer by ALD method, improves corrosion resistance. Correct selection of parameters for applying the layer also resulted in adequate adhesion to the base, which effectively minimizes the migration of ions of the elements Fe, Cr, Ni or Mo.

References

[1] A. Nouri, C. Wen C.: Introduction to surface coating and modification for metallic biomaterials. Surface Coating and Modification of Metallic Biomaterials, Elsevier, 2015.
[2] Q. Zhong et al.: Atomic layer deposition enhanced grafting of phosphorylcholine on stainless steel for intravascular stents, Colloids and Surfaces B: Biointerfaces, 121 (2014) 238–247.
[3] E. Zalnezhad, A. M. S. Hamouda, G. Faraji, S. Shamshirband, TiO_2 nanotube coating on stainless steel 304 for biomedical applications. Ceramics International 41 (2015) 2785–2793.
[4] W. Walke, Z. Paszenda, M. Basiaga, P. Karasiński, M. Kaczmarek: EIS Study of SiO_2 Oxide Film on 316L Stainless Steel for Cardiac Implants, In: E. Piętka, J. Kawa, W. Wieclawek W. (eds) Information Technologies in Biomedicine, Vol. 4. Advances in Intelligent Systems and Computing, 284. Springer, Cham, 2014.

FORMATION OF FUNCTIONAL COATINGS ON Ti-6Al-7Nb ALLOY SURFACE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 82]

Introduction

Selected titanium alloys, such as Ti-6Al-7Nb, Ti-15Mo, exhibit mechanical properties close to natural bone. The corrosion products of these Ti alloys are non-toxic and non-allergic for human organism [1]. Surface of titanium alloy is modified to obtain multifunctional material for bone replacement or regeneration [2]. Plasma electrolytic oxidation (PEO) is easy and cost-effective methods of surface treatment [3]. The one advantage of the PEO is possibility of anodization of various shapes Ti implants. Microstructure and chemical composition of porous oxide layer might be design to obtain materials which enhance osseointegration process [4]. The long-term exposure of the implant surface in organism may be complicated by infection, with pathogenic bacteria, adhering to the implant [5]. To prevent bacterial biofilm formation, the porous oxide layer is cover by thin polymer layer with biological active substance [6]. Drug release into surrounding tissue might be controlled by time degradation of polymer deposited on previously anodized surface. One of the biocompatible polymer which exhibit degradation in artificial saliva is poly(D,L-lactide-co-glycolide.

Materials and Methods

Surface of Ti-6Al-7Nb alloy was anodized in solution composed of $Ca(H_2PO_2)_2$ and $CaSiO_3$ at 350V. Time of surface treatment was 5 min, when current density was 150 mA/cm^2. On the porous oxide layer the biodegradable polymer (poly(D,L-lactide-co-glycolide-PLGA was deposited using dip coating method.
Hybrid, oxide-polymer coatings were characterized using scanning electron microscope (SEM, Phenom ProX), Raman spectroscopy with CDD detector. Degradation of polymers layer in artificial saliva was evaluated using ^{1}H NMR technique.

Results and Discussion

FIG. 1. presents representative SEM images of the anodized Ti-6A-7Nb surface and after additional surface treatment using biodegradable polymer.

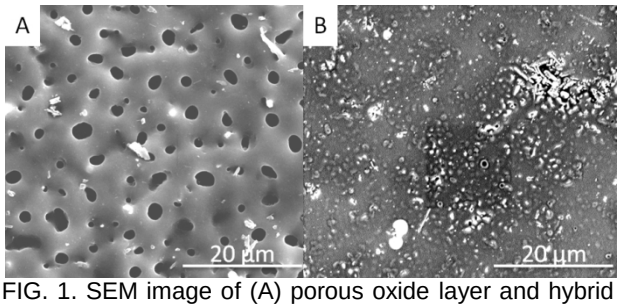

FIG. 1. SEM image of (A) porous oxide layer and hybrid oxide-polymer layers formed on Ti-6Al-7Nb alloy surface. Magnification: x2000.

PEO process caused that the coating was porous. Incorporated particles of wollastonite were clearly visible on the surface. After dip coating, pores of the oxide layer were filled by biodegradable polymer. However, still some wollastonite particles were visible on the top of the surface, which may indicate that the polymer layer wasn't too thick. Product of polymer degradation was detected by ^{1}H NMR technique, after 4 weeks of sample immersion in artifiial saliva. Results indicated that the polymer layer degradated in very short time.
FIG. 2. presents Raman spectra of the hybrid layer formed on the Ti alloy surface.

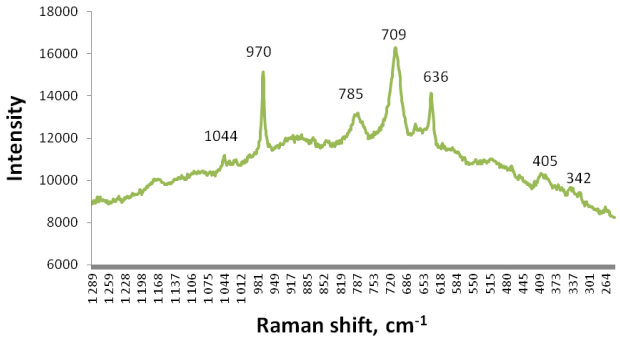

FIG. 2. Raman spectra of the hybrid layer formed on the Ti alloy surface.

The Si-O stretching signals at 636 cm^{-1} and 970 cm^{-1} were from wollastonite phase. The Ca-O stretching modes from the wollastonite was detected at 405 cm^{-1}. Signal at 342 cm^{-1} corresponds to TiO_2 anatase phase, when signals at 709 cm^{-1}, 785 cm^{-1} and 1044 cm^{-1} were detected for PLGA layer formed on anodized Ti surface.

Conclusions

Possibility of the anodization of Ti-6Al-7Nb surface allows design material which enhances integration of the implant surface with bone tissue. The polymer layer which was deposited on the porous oxide layer might be enrich with selected drugs such as doxycycline, vancomycin, gentamicin or clindamycin. Proposed surface treatment of the Ti alloy might exhibit antibacterial properties against bacteria strains such as: S. aureus, E. coli strains and coagulase-negative staphylococci (CoNS) as the most common infections pathogens.

Acknowledgments

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This work was supported by the Silesian University of Technology, Gliwice Poland, Rector's grant no. 04/010/RGJ17/0049.

References

[1] B.M. Holzapfel, J.C Reichert, *et al.,* Adv. Drug Delivery Rev. 63 (2013) 581-603.
[2] R. Iona, D.-M. Gordin, *et al.,* Mater Sci Eng C 35 (2014) 411-419.
[3] I. Apachitei, A. Leoni, *et al.,* J. Appl. Surf. Sci. 257 (2011) 6941-6944.
[4] B. Yang, M. Uchida, *et al.,* Biomaterials 25 (2004) 1003-1010.
[5] Z.H. Baqain, W.Y. Moqbel, *et al.,* Brit J. Oral Max. Sur. 50 (2012) 239-243.
[6] A. Kazek-Kęsik, J. Jaworska *et al.,* Surf. Coat. Technol. 30 (2016) 158-165.

CHARACTERIZATION OF GRAPHENE OXIDE-LOADED CHITOSAN HYDROGELS AND THEIR APPLICATION FOR 3D PRINTING

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[ENGINEERING OF BIOMATERIALS 143 (2017) 83]

Introduction

Nowadays, additive manufacturing methods, and 3D printing especially, are widely applied in numerous fields from automotive and aerospace to biomedicine and tissue engineering[1]. In the latter, this technique is used e.g. for fabrication of TE scaffolds from variety of biodegradable polymers. Among them, hydrogels seem to be one of the most promising due to their high water content and ability to mimic microenvironment of a natural extracellular matrix (ECM)[2]. Hydrogels possess excellent biological properties but their printability might be challenging, as – when printed - those relatively soft structures tend to collapse under the weight of subsequent layers. To print hydrogel scaffolds in a highly precise manner, it is necessary to have appropriate gel ink viscosity and homogeneity. From the biological perspective, the most important is the biocompatibility of the scaffold material. From the variety of materials of natural origin, chitosan is of particular interest thanks to its antibacterial, mucoadhesive, hemostatic, analgesic, and antioxidant properties, as well as excellent biocompatibility and biodegradability.
In this work, chitosan and chitosan/graphene oxide hydrogels were designed and optimized for 3D printing. Investigated parameters included solvent type and solution concentration, gel viscosity, printing pressure, feed rate, needle gauge, and post-treatment with e.g. sodium hydroxide.

Materials and Methods

Chitosan (CS, High Mw, Sigma-Aldrich) and chitosan/GO inks for printing were prepared by dissolving the chitosan (5% w/v) in 10% lactic acid or mixture of lactic acid and gallic acid. Next, different amounts of stable GO suspension in distilled water were added to CS solution (0%, 0.5% and 1% to CS weight). At each step, the solutions were homogenized thoroughly by vortexing and sonication in a water bath. The prepared inks were transferred to a special syringe and centrifuged to remove remaining air bubbles. Hydrogel inks were printed using 3D-Bioplotter® (Envisiontec, Germany) (STL files created in SketchUp 2016 3D modelling software). After drying, some of the scaffolds were post-treated by neutralization in 1M NaOH solution.
Rheological properties of the hydrogel inks were examined using rotational Kinexus rheometer with a parallel plate geometry (Malvern Instruments Ltd, UK). Also, ATR-FTIR Nicolet™ iS™, Thermo Fisher Scientific) spectroscopy and optical microscopy were used to characterize the samples. Fibroblasts viability was assessed with Alamar Blue assay.

Results and Discussion

Rheological characterization confirmed that chitosan-based hydrogel inks are non-newtonian fluids and exhibit shear-thinning behavior – with increasing shear stress, the fluid viscosity decreased (FIG. 1). Rheological properties of chitosan depend on the solution concentration. Addition of graphene oxide to the chitosan matrix resulted in the increase of the gel viscosity.

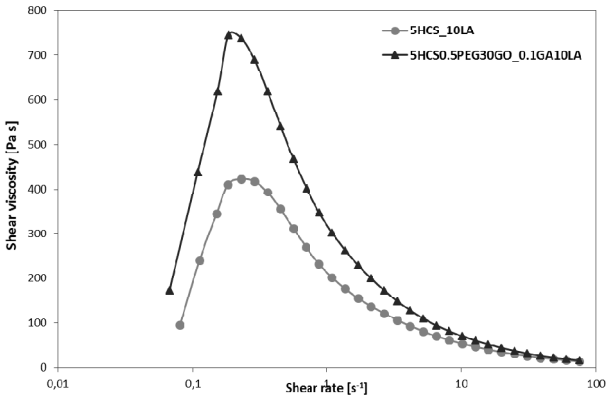

FIG. 1. Viscosity vs. shear rate for chitosan (dots), and chitosan/GO (triangles) inks.

The best printing resolution was achieved with 5% (w/v) chitosan solution modified with graphene oxide. Optimized printing parameters were as follows: 25 gauge dispense tip (Nordson EFD), pressure 5 bar, speed 1.5-2 mm/s, at room temperature. Thanks to relatively high concentration of the gel ink and the low feed rate, remaining solvent could partially evaporate, hardening the printed layer and securing enough structural support to avoid collapsing of the hydrogel struts under the weight of another layer. Printed scaffold could be easily manipulated using tweezers. Some drying-induced shrinkage was observed, however in general, scaffolds remained their designed morphology. When immersed in water, dried chitosan scaffolds swelled and dissolved rapidly; dried and then neutralized scaffolds, after some initial swelling, remained stable. Interestingly, also dried-only CS/GO composite scaffolds were stable in the aqueous environment. It is possible that graphene oxide sheets crosslinked chitosan network, increasing its stability.

Conclusions

Chitosan and chitosan/GO composite hydrogels can be 3D printed creating scaffolds with predefined architecture. Appropriate ink preparation is crucial for successful printing. Higher gel viscosity results in better printing resolution.

Acknowledgments

Dr. Joseph A.M. Steele at Kohn Lab is gratefully acknowledged for his mentoring and assistance with 3D printing. This research was supported by the New Jersey Center for Biomaterials, Rutgers University (through ICF-BSE International Summer Exchange Program), and by the grant No 15.11.160.019 (Faculty of Materials Science and Ceramics, AGH University of Science and Technology).

References

[1] Y. He, F. Yang, *et al.*, Sci. Rep. 6 (2016) 29977.
[2] M. Guvendiren, J. Molde, *et al.* ACS Biomater. Sci. Eng. 2016, 2, 1679−1693.

MODELING, FABRICATION AND CHARACTERIZATION OF 3D PLLA SCAFFOLDS PLOTTED WITH COMMERCIAL PRINTER

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[ENGINEERING OF BIOMATERIALS 143 (2017) 84]

Introduction

Biomedical application is one of the main areas of 3D printing usage specifically in creating scaffolds for medical implants [1-2]. Scaffolds made of PLLA can easily mimic the structure, shape, pore size and topography of natural tissue. The use of a three dimensional CAD model in RP allows the recreation of complex 3D architecture, mechanical properties and composition of the materials in contact with cells [3-6]. Supported on the computed tomography (CT) scans RP enables the exact reproduction of natural tissues [7]. The main purpose of this work was to fabricate scaffolds using commercial 3D printer. Different scaffold topography and geometry was created using 3D modelling software.

Materials and Methods

The fabrication process begins in the 3D surface modeller (Rhino3D v5) where scaffold prototype model was obtained. Afterwards 3D model was transferred to 3D FDM printer as -.STL file. The prototype had lattice form made of five layers (FIG. 1a) created by bars with 1 mm rectangular cross-section and 1 mm spacings. Each layer was rotated at 90º with respect to previous one. The dimensions of the scaffold were length 21 mm (11 bars in layer 2, 4), width 9 mm (5 bars in layers 1, 3, 5), height 5 mm.

Poly L-lactic acid (PLLA, 3D4Makers) with a diameter of 1.75 mm, molecular weight 60000-80000 Da, and viscosity of 300000 CP was used as filament for 3D printer (Zortrax M200, Poland). A PLLA filament was fused and guided by an extrusion nozzle to form 3D scaffolds. Printer nozzle temperature was 210°C and the height of printed layer was set to 0.09 mm. The material left the extruder in a liquid form and solidifies upon contact with the fabrication platform (temp. range: 20-60°C). The previously formed layer was the substrate for the next layer.

The samples were evaluated with Stereomicroscope (SN from OPTA-TECH company, equipped with CMOS 3 camera and OptaViewIS software) and with scanning electron microscope (Nova NanoSEM 200, FEI) equipped with EDS analysis. Tensile strength Rm was determined in a static tensile test on Hegewald und Peschke testing machine (Inspekt Table Blue 5kN).

Results and Discussion

Using FDM - additive fabrication process (FIG. 1b - clearly seen each deposited layer of material) it was possible to fabricate the PLLA scaffolds with commercial 3D printer. The selected pattern was simple but provided easy modelling and rather high number of inner and outer surfaces that constitutes places where the cells can grow (FIG. 1c,d). Microstructure and mechanical properties of obtained scaffold were evaluated. The properties of the lattice microstructure of designed scaffold were compared with relevant one for solid cuboid (TABLE 1). Future experiments will be also undertaken to evaluate formation of apatite in Simulated Body Fluid (SBF).

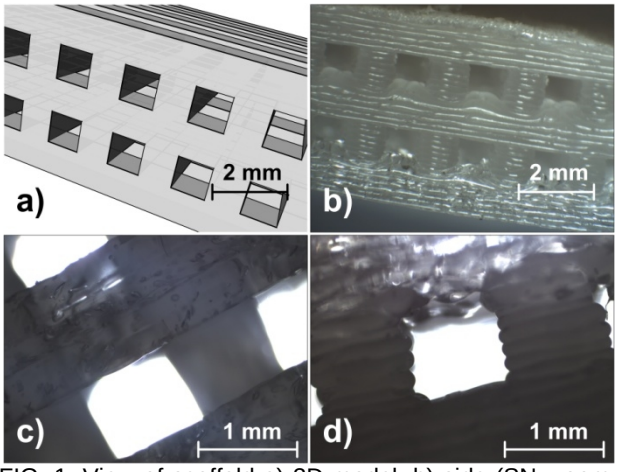

FIG. 1. View of scaffold a) 3D model, b) side (SN, zoom 8x), c) top (MB 200, 40x), d) side (MB 200, 40x).

TABLE 1. The comparison of scaffold properties for lattice microstructure and solid cuboid.

Microstructure	Volume [mm^3]	Porosity [%]	Total Area [mm^2]
Lattice structure	513	45.7	1686
Reference cuboid	945	0	678

Conclusions

Three-dimensional (3D) printers can create complex structures based on digital models. Our preliminary research show the potential of using the commercial printers for plotting PLLA scaffolds with designed geometry and thus influence the scaffold mechanical properties.

Acknowledgments

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References

[1] B. Derby, Science 338 (2012) 921–926.
[2] S. Yang, K.-F. Leong *et al.*, Tissue Eng. 8 (2002) 1-11.
[3] K.K. Gómez-Lizárraga, C. Flores-Morales *et al.*, Materials Science and Engineering C (79) (2017) 326-335.
[4] S. Giannitelli, D. Accoto *et al.*, Acta Biomater. 10 (2) (2014) 580-594.
[5] D.W. Hutmacher, M. Sittinger *et al.*, Trends Biotechnol. (22) (2004) 354-362.
[6] E. Sachs, M. Cima *et al.*, CIRP Ann. Manuf. Technol. (39) (1990) 201-204.
[7] R. Landers, R. Mülhaupt, Macromol. Mater. Eng.282 (2000) 17-21.

ANTIOXIDANT PROPERTIES OF BIOACTIVE FOOD PACKAGING WITH NANODIAMONDS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 85]

Introduction
The high biological activity of nanodiamond particles is based on the reactivity of diamond surface that results from its crystallographic structure and the presence of free bonds. Nowadays, materials for food packaging are a new intriguing challenge for scientists. The impact of material for food packaging in addition to the effect on the bacterial flora and the shelf life caused the search for new materials, which particularly are non-toxic and bioactive [1].

Materials and Methods
Corona treatment is a surface modification technique that uses a low temperature corona discharge plasma to impart changes in the properties of a surface. We incorporated detonation nanodiamond particles into polymer food packaging foil.
The measurements contains: the sandard packaging for butter (82 % of fat) and ND-incorporated packaging.
The p-anisidine value (AV) is a measure of the amount of lipid oxidation secondary products. In good oils and fats acceptable value is AV <2. Measurements for both samples were made three times out of three individually prepared samples [2].

Results and Discussion
The study showed the antioxidant effect of the modified ND package. The p-anisidine value in the case of butter in the original packaging is twice as large as in the modified packaging LA for butter in original packaging:
LA = 0.5667 ± 0.022LA for the sample in the modified packaging LA = 0.2735 ± 0.018.

Conclusions
Bioactive food packaging with incorporated nanodiamonds particles has antioxidant properties.

References
[1] Mitura, K., Zarzycki, P., Biocompatibility and toxicity of allotropic forms of carbon in food packaging, 2017, in: A.M. Grumezescu (Eds.), Food Materials Science, Volume 19, ELSEVIER, in press.
[2] Subramanian, R., Nandini, K.E., Sheila, P.M., Gopalakrishna, A.G., Raghavarao, K.S.M.S., Nakajima, M., Kimura, T. & Maekawa, T. (2000) Membrane processing of used frying oils. J. Am. Oil Chem. Soc., 77, 323-328.

USE OF DIAMOND POWDER IN THE IONIZATION PROCESS

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[ENGINEERING OF BIOMATERIALS 143 (2017) 86]

Introduction
Iontophoresis, also known as ionization, is a medical treatment with the use of electric current. It consists in introducing ions into the epidermis and uses the electrolytic dissociation phenomenon, that is the separation of molecules into positively and negatively charged ions. Iontophoresis transports small molecules of active substances in low current electric field and introduces substances in the form of ions into the skin.

M**aterials**
Diamond powder with a diameter of 2-4 nanometers.

Method
The use of direct current increases the ND penetration. Electro osmosis leads to larger ND ions diffusion into the epidermis. Ions, after penetrating stratum basale epidermis keep diffusing into the deeper layers of epidermis for 24-48 hours. After the procedure the explicit biocompatibility of ND was ascertained.

Results and Discussion
The process of iontophoresis enabled answering the question of ND's biocompatibility. After the procedure several pictures were taken with video dermatoscope before and after the usage of ND. Skin penetrations reach the deeper layers of the epidermis. The diamond powder has an antioxidant effect and is biocompatible.

Conclusions
After the first visual clinical evaluation of the skin, no contact lesions were observed. After exposing the preparation to the skin, no change in the immune mechanism was demonstrated**.**
The additional measurable aspect was high hydration level of epidermis after the procedure. The measurement was made with the use of corneometer.

Acknowledgments
Acknowledgment for Professor Katarzyna Mitura for help with the implementation of the research.

References
[1] K. Mitura, P. Niedzielski, G. Bartosz, J. Moll, B. Walkowiak, Z. Pawłowska, P. Louda, M. Kieć-Świerczyńska, S. Mitura: „Interactions between carbon coatings and tissue", Surface and Coatings Technology, 201, (2006), 2117-2123.
[2] D. Bociąga, J. Grabarczyk, P. Niedzielski, M. Krakós, K. Mitura: „Application of biomaterials with Carbon Coatings for Body Piercing", Engineering of Biomaterials, 43-44 (2005) 66-70.

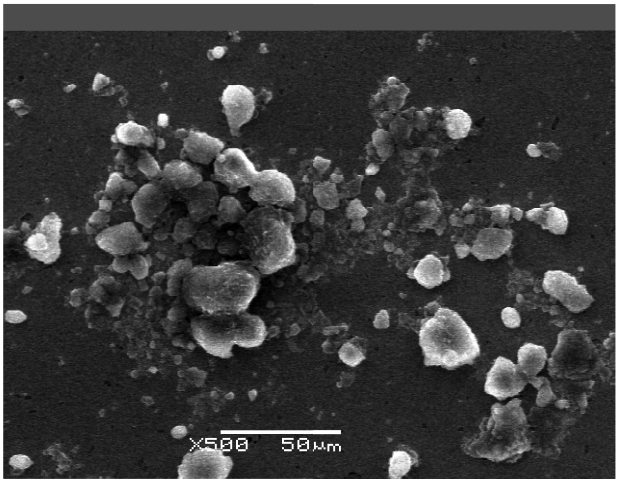

FIG. 1. SEM image of three-dimensional (3-D) structure of detonation nanodiamond particles (JEOL JSM-5500LV).

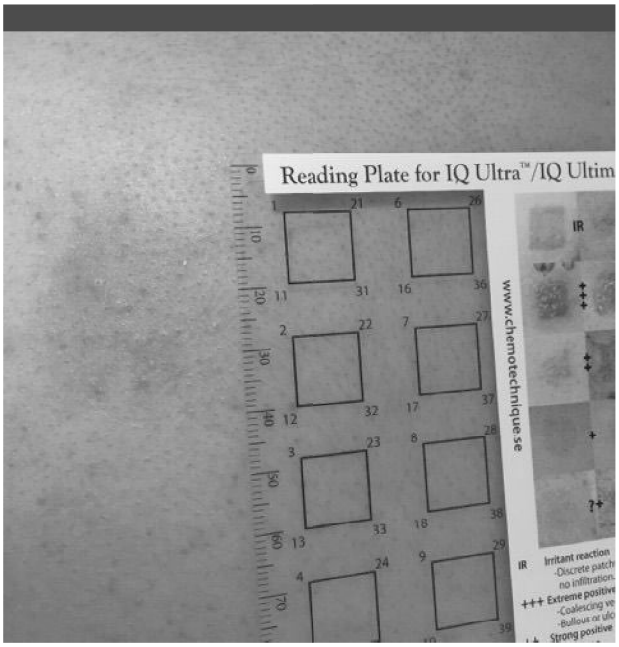

FIG. 2. Allergic reaction to cathode.

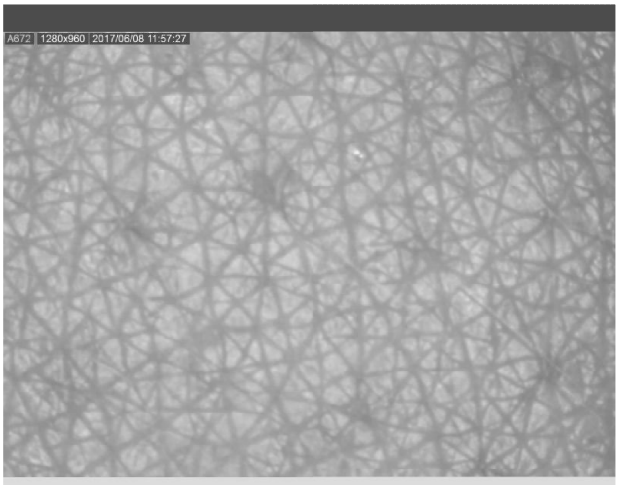

FIG. 3. No allergic reaction.

USE OF NANO-DIAMOND IN COSMETIC MICRODERMABRASION

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[ENGINEERING OF BIOMATERIALS 143 (2017) 87]

Introduction

Microdermabrasion consists of controlled mechanical abrasion of the epidermis. The intensity of the surgery depends on the degree of diamond gradation, vacuum used in the device, head shape, time and speed action and pressure. Microdermabrasion affects appearance epidermis and many changes that occur in the skin. Exfoliating the outer layers of the epidermis, purifies the skin with excess levels keratinocytes, sebum. Also narrows the sebaceous gland, smoothes out cuticle and gradually reduce the depth of the scar and reduce the visibility of wrinkles.

Materials and Methods

Materials

USG gel

Diamond powder without modifications with a diameter of 2-4 nanometers.

Methods

The preparation was based on gel for USG in a ratio of 1 mg nano diamond powder without modifications per ml of gel.

Microdermabrasion is a procedure in which a smoothly moving head is covered with diamond microcrystals. The skin is sucked trough the head by the vacuum produced by the device.

Results and Discussion

None of the had hypersensitivity reactions. What is more, no dermograph was observed.

The post-operative erythema was more rapidly resorbed by the ND application. Lack of response and irritation reaction (IR) and allergic reaction of AR [1-3].

Conclusions

Faster recovery of erythema from irritation is a result of ND.

Acknowledgments

Katarzyna Mitura and Marek Chuchracki thank you for your substantive support and help in research.

References

[1] M. Batory, D. Batory, J. Grabarczyk, W. Kaczorowski, B. Kupcewicz, K. Mitura, T.H. Nasti, N. Yusuf, and P. Niedzielski, „Biological Properties of Carbon Powders Synthesized Using Chemical Vapour Deposition", Journal of Nanoscience and Nanotechnology, 12(12), (2012), 9037-9046.

[2] V.V. Danilenko, On the history of the discovery of nanodiamond synthesis. Phys. Solid. State. 46, (2004), 595–599.

[3] B. Kręcisz, M. Kieć-Świerczyńska, M., K. Bąkowicz-Mitura, Allergy to Metals as a Cause of Orthopedic Implant Failure. Int. J. Occup. Med. Env. 19(3), (2006), 178-180.

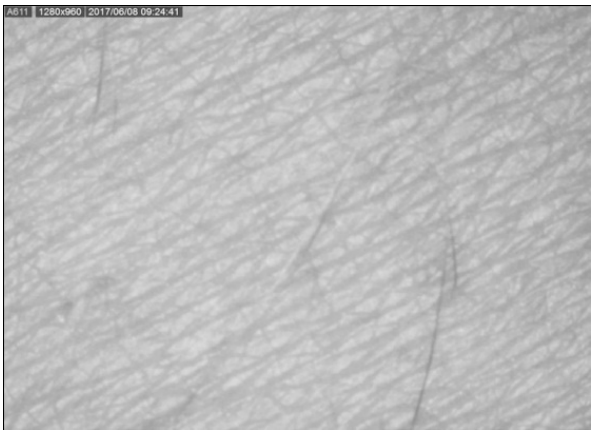

FIG. 1. Skin before the microdermabrasion surgery.

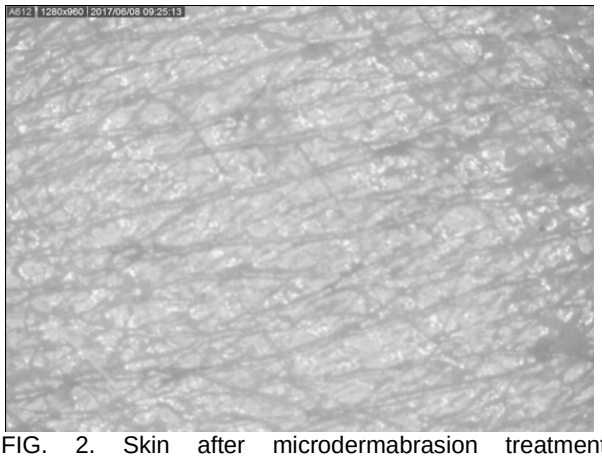

FIG. 2. Skin after microdermabrasion treatment, ND application with USG gel (1 ml per 1 mg).

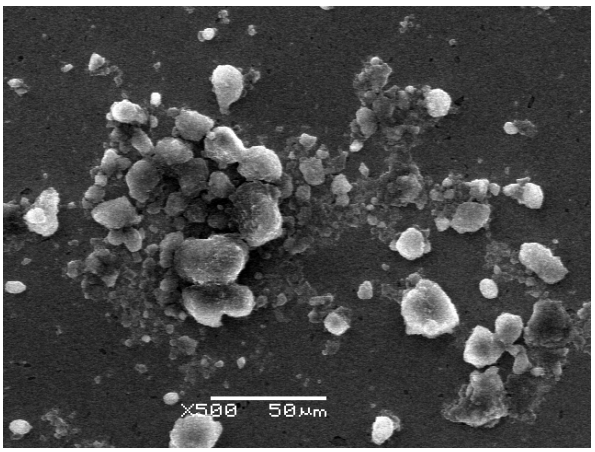

FIG. 3. Image of three-dimensional (3-D) structure of detonation nanodiamond particles (JEOL JSM-5500LV).

TEMPERATURE DEPENDENCE OF CYCLIC BEHAVIOR OF POLY (LACTIC ACID)/HYDROXY-APATITE NANOCOMPOSITES

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[ENGINEERING OF BIOMATERIALS 143 (2017) 88]

Introduction

PLA is a biodegradable and biocompatible polymer widely used in biomedical applications for fracture fixation, interference screws, suture anchors and meniscus repair [1]. To enhance mechanical and physical properties of PLA (which has rather modest elastic moduli and strength and demonstrates brittleness at ambient temperature [2]), it is melt-blended with polymers with high tensile and impact strength [3], chemically modified with plasticizers, toughened with impact modifiers [4], and reinforced with microparticles and nanoparticles [5]. Although mechanical properties of PLA and composites with PLA matrices have been analyzed in a number of studies, a thorough examination of the influence of temperature and loading rate has not yet been performed [6]. This study focus on experimental investigation of the cyclic behavior of poly(lactic acid) (PLA)/hydroxyapatite (HA) composites under tension in the interval of temperatures from room temperature to 50ºC at cross-head speeds of 1 mm/min. Observations show that the presence of filler leads to a slight increase in elastic modulus and maximum stress at 1.5% strain level and induces slightly growth of recovery up to 1 MPa stress level. Given a filler content, maximum stress at 1 MPa decreases with temperature.

Materials and Methods

Poly(lactic acid) Polymer 4042D (density 1.24 g/cc) was purchased from Nature Works LLC (USA). Biphasic synthetic Hydroxyapatite (70% (HA)/Beta Tri Calcium Phosphate (30% (βTCP)) ceramics powder BioGraft (particle size 0.37 μm) was supplied by Ifgl Bio Ceramic Ltd (India). Ceramics powder was mixed with PLA in proportion corresponding to 5 wt%. Dumbbell specimens for tensile tests (ASTM standard D-638) were molded by using injection-molding machine Arburg 320C. To assess glass transition and melting temperatures, DSC (differential scanning calorimeter) measurements were performed by means of Mettler Toledo DSC 823E apparatus at heating rate 20 K/min under nitrogen flow. Glass transition and melting peaks of PLA and PLA/HA composite equal T_g = 63ºC, T_m = 155ºC, and T_g = 68ºC, T_m = 157ºC, respectively. Specific enthalpies of melting read 10.2 J/g and 19.1 J/g, which corresponds to degrees of crystallinity 11 and 21%. Mechanical tests were conducted by means of universal testing machine Instron-5568. The specimens are loaded with a constant strain rate up to 1.5% strain and unloaded with the same strain rate up to 1 MPa stress with cross-head speeds 1 mm/min at temperatures 23, 30, 35, 40, 45 and 50ºC.

Results and Discussion

Experimental stress-strain diagrams of PLA and PLA/HA composite are depicted in FIG. 1. The mechanical behavior of PLA/HA composites is brittle at room temperature and becomes ductile at elevated temperatures. Maximum stress at 1.5% of PLA/HA is slightly higher than that of PLA and decreases with temperature. Maximum stress at 1.5% decreases strongly with temperature. Unloading behavior of PLA/HA at high temperature level are nonlinear. Viscoelastic recovery after unloading decreases with an increase in temperature.

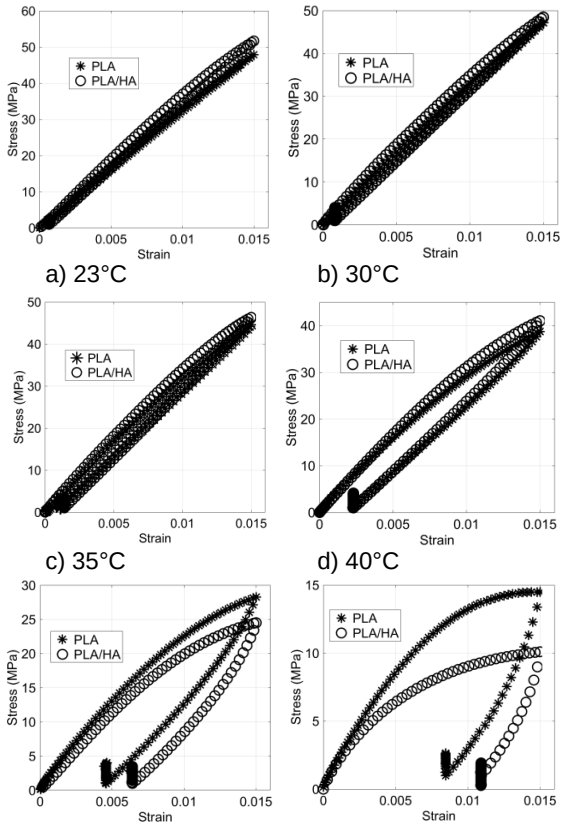

FIG. 1. Comparison of cyclic behavior of PLA and PLA/HA.

Calculated maximum stress and recovered strain of PLA and PLA/HA nanocomposite are given in TABLE 1.

TABLE 1. Maximum stress and recovered strain.

σ_{max} at 1.5%	Temp.°C	23	30	35	40	45	50
	PLA	47.9	47.1	44.6	41	28.4	14.4
	PLA/HA	51.7	48.4	46.4	38.7	24.5	10.1
%recovered strain at 1 MPa							
	PLA	1.43	1.41	1.38	1.27	1.04	0.65
	PLA/HA	1.43	1.41	1.39	1.27	0.86	0.4

Conclusions

A thorough investigation is performed of the effects of temperature on the cyclic behavior of PLA/HA composites under uniaxial tension. Reinforcement of PLA with HA induces a slight increase in elastic modulus and a decrease in maximum stress at 1.5% strain. Loading–unloading behaviors of PLA/HA are nonlinear and temperature dependent. Viscoelastic recovery after unloading decreases with increasing temperature.

References

[1] K.V.Velde and P. Kiekens, Polym. Test. 21 (2002) 433.
[2] P.F. Carfi, S. Rigogliuso, C.V. La, G.A. Mannella, G. Ghersi, and V. Brucato, Chem. Eng. Trans. 27 (2012) 409.
[3] JG. Stoclet, R. Seguela, and J.-M. Lefebvre, Polymer 52 (2011) 1417.
[4] H. Liu and J. Zhang, J. Polym. Sci. Part B: Polym. Phys. 49 (2011) 1051.
[5] P. Russo, D. Acierno, A. Vignali, and M. Lavorgna, Polym. Compos. 35 (2014) 1093.
[6] N. Dusunceli, A.D. Drozdov, N. Theilgaard, Polym. Eng. Sci. 57 (2017) 239.

MECHANICAL PROPERTIES OF COMPOSITE SCAFFOLDS FROM POLY(3-HYDROXYBUTYRATE) AND SODIUM ALGINATE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 89]

Introduction

Recent years biodegradable polymers of natural origin, poly(3-hydroxyalkanoates) (PHAs) and alginates (ALGs), have found broad application in medicine and tissue engineering. These polymers are very different in their properties: PHAs are hydrophobic, mechanically strong polyesters, while alginates are hydrophilic, hydrogel-forming, mechanically destructible polysaccharides. However these polymers bring together the fact that PHAs and ALGs can be produced biotechnologically allowing to regulate their properties [1,2]. Particularly, development of composite constructions from these polymers makes it possible to adjust the selected properties, especially mechanics, of the resulting composite PHAs/ALGs constructions for bone and cartilage engineering, where PHAs and ALGs are widely used. Thus, the objective of the work was to create the composite scaffolds from poly(3-hydroxybutyrate) (PHB) and sodium alginate (ALG).

Materials and Methods

Two types of PHB/ALG constructions were manufactured: porous constructs from PHB filled with ALG hydrogel (ALG-in-PHB) and ALG hydrogel embedded with PHB microspheres (PHB-in-ALG). The PHB porous constructs used in this work were manufactured by two-stage leaching technique using two blowing agents: ammonium carbonate and sucrose and then filled with ALG with hydrogel formation. PHB microspheres were produced by two-stage emulsification technique and then mixed with ALG to produce hydrogel. Various PHB/ALG composite construction with different parameters: pore size, microspheres diameter, microspheres content, hydrogel density were produced. The morphology of composite scaffolds was investigated by scanning electron microscopy (SEM) and by wide-field light microscopy (WLM). The mechanical properties of obtained constructions were measured by rheometry.

Results and Discussion

The Young's modulus of obtained PHB/ALG composite scaffolds varied from 9 to 178 kPa. The complicated dependence between mechanical properties and morphological features of PHB/ALG composite scaffolds was revealed and analysed. Morphological features (e.g. pore size, microspheres diameter, porosity) of produced scaffolds effect greatly on its mechanics: e.g. increase in diameter of microspheres from 50 to 500 mkm caused 6-fold increase in Young's modulus of PHB-in-ALG scaffolds.

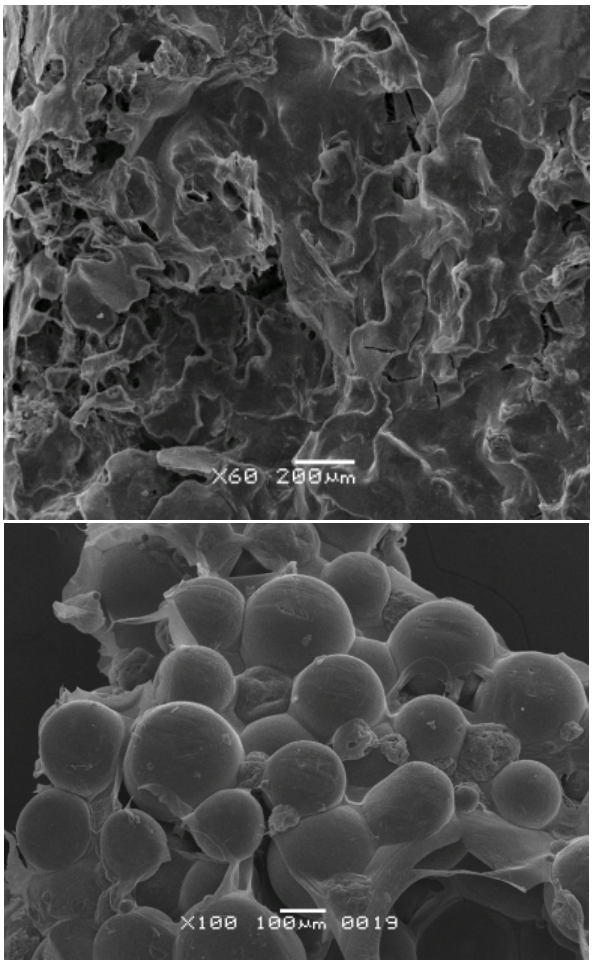

FIG. 1. SEM microphotographs of ALG-in-PHB (a) and PHB-in-ALG scaffolds (b).

Conclusions

In general, PHB microspheres reinforced PHB-in-ALG scaffolds more efficiently than PHB porous structures ALG-in-PHB scaffolds. Further the technique of hybrid PHB/ALG scaffolds production will be used to develop biocomposite scaffolds and fillers for bone tissue engineering.

Acknowledgments

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References

[1] A. Bonartsev, I. Zharkova *et al.,* Preparative Biochemistry and Biotechnology, 47 (2017), 173-184.
[2] G. Bonartseva, E. Akulina *et al.,* Applied Biochemistry and Microbiology, 53 (2017), 51–58.

ARBURG Plastic Freeforming

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[ENGINEERING OF BIOMATERIALS 143 (2017) 90]

Customised medical devices and implants
Industrial additive manufacturing from the perspective of a machine manufacturer: this requires a practical approach involving many new solutions – and an innovative, patented process - ARBURG Plastic Freeforming (APF). In other words: a completely new way of working! Additive manufacturing. With standard granulate and layers built up from tiny droplets of plastic.

Our innovative APF process enables you to combine design freedom with a wide choice of materials. This enables you to quickly produce replacement parts or functional samples from the desired material, for example. Whatever industry you may work in, the freeformer offers you a wide range of new options: from consumer goods and automotive parts to medical technology - to name just a few examples.

Especially resorbable materials like PLLA would be destroyed just by making a wire of it… And you can use already NDA approved pellets use for any implant. Also important in the medical field: one mould is needed for one part… And as a bonus you just purge a few grams of material in the freeformer instead of making filling trial in the ALLROUNDER. In this range of material prices, it can make the difference…

The topic of Industry 4.0 directly affects us, our customers and the entire plastics industry. Those who prepare for the future with flexible, automated production technology and IT networking will secure and develop the competitiveness of their companies for years to come. However, this calls for interdisciplinary expertise. ARBURG is therefore particularly well positioned in this area: with automated ALLROUNDERs, the central SELOGICA control system, the freeformer for industrial additive manufacturing and IT solutions such as the ALS host computer system, ARBURG offers a full range of expert skills. This enables you to implement your individual 4.0 solutions in a targeted way and to increase your production efficiency in a sustained manner.

APF – more than just 3D printing:

- Open system – Build up your own know-how
- Making plastic products using standard granulate
- Broad range of process able materials
- Individual process settings
- High-grade mechanical properties
- Efficient use of materials

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FIG. 1. Individually adapted implant for cranial bones was produced in the APF process using medical grade PLA granulate (Resomer).

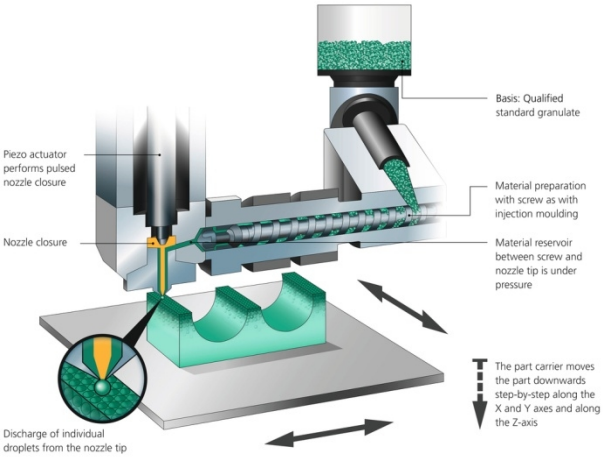

FIG. 2. The APF process principle in detail.

FIG. 3. Freeformer: Open production system for the APF process.

STED AND SEEC IMAGING - NEW SOLUTIONS FOR BIOSCIENCE

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[ENGINEERING OF BIOMATERIALS 143 (2017) 91]

Introduction

We want to introduce new SEEC N-Lab superresolution research station for Life Science and show STED solutions from Abberior Instruments GmbH. Compare both above with light sheet microscopy. In addition few words about other devices available from for NSOM, TERS, PALM, STORM and FLIM.

Materials and Methods

Typical applications and potential fields of use for above products will be presented. Selected articles where our equipment was used will be listed as well as sample images showing achievable image resolution for each technique.

Other Products

In addition to superresolution imaging devices we also offer other products for bio-related research. Those products include:

- BioAFM Hydra and nc-AQUA AFM
- BioXolver automated SAXS station
- Laboratory LIBS elemental analyzer
- µXRF AttoMap system from Sigray
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