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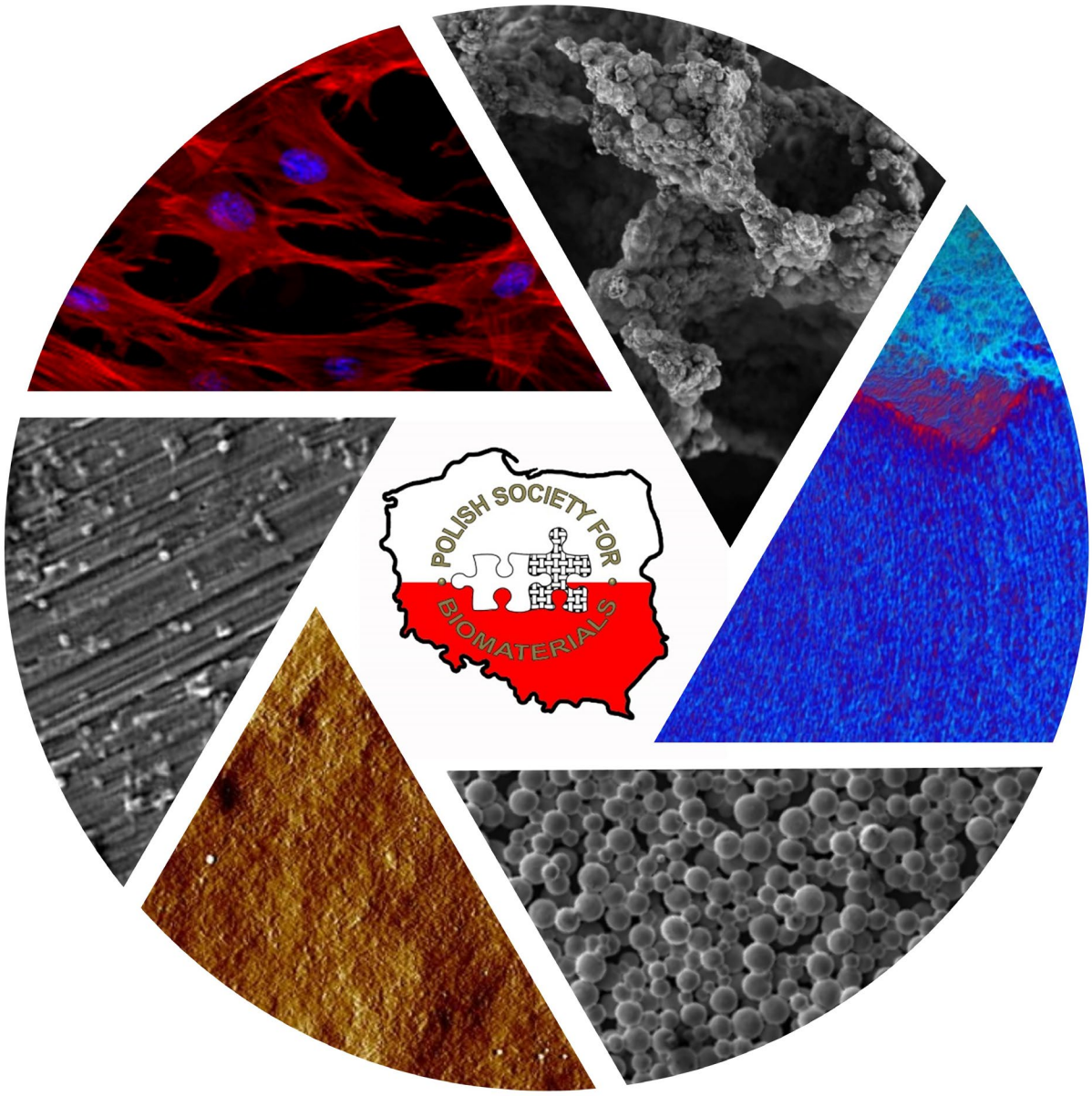

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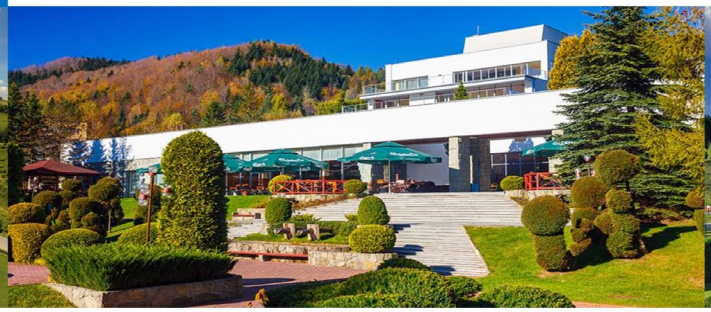

CONTENTS

25. **THE BACTERICIDAL DEGRADATION EFFECTIVENESS OF TANNIC ACID-BASED THIN FILMS FOR WOUND CARE**
Beata Kaczmarek, Marcin Wekwejt, Kinga Nadolna, Agata Owczarek, Olha Mazur, Anna Pałubicka

26. **PILOT STUDIES OF BIODEGRADABLE IRON-BASED 3D SYSTEMS – FOR THE NEEDS OF MODERN CARDIOLOGY**
Aleksandra Radtke, Jonasz Szczepański, Piotr Piszczek

27. **THE ANTIMICROBIAL AND PHOTOCATALYTIC ACTIVITY EVALUATION OF THE POLYMER/TITANIUM(IV) OXO-COMPLEX COMPOSITES**
Barbara Kubiak, Aleksandra Radtke, Patrycja Golińska, Piotr Piszczek

28. **DUAL CROSS-LINKING AS A METHOD OF IMPROVING MECHANICAL PROPERTIES OF GELATIN-ALGINATE HYDROGELS**
Marta Tuszyńska, Joanna Skopińska-Wiśniewska

29. **HYDROGEL-BASED MATERIALS FOR DELIVERY MEDICINAL PLANT EXTRACTS – PRELIMINARY STUDIES**
Justyna Kozlowska, Marta Sienkiewicz, Anna Lipinska

30. **PREPARATION AND CHARACTERIZATION OF BIO-HYBRID HYDROGEL MATERIALS**
Dagmara Malina, Katarzyna Bialik-Wąs, Klaudia Pluta

31. **DESIGN AND MANUFACTURE OF CUSTOMIZED MEDICAL IMPLANTS - THIRD REPORT**
Marcin Elgalal, Piotr Komorowski, Krzysztof Makowski, Andrzej Styczyński, Piotr Ulański, Tomasz Kubiak, Jacek Sawicki, Marcin Domżalski, Bogdan Walkowiak

32. **MAY METALLIC BIOMATERIALS USED FOR ORTHOPEDIC IMPLANTS PROMOTE CANCER PROCESSES? PRELIMINARY TRASRIPTOMICS RESEARCH ON HUMAN ARTICULAR CHONDROCYTES.**
Magdalena Walkowiak-Przybyło, Marta Walczyńska, Marta Kamińska, Piotr Komorowski, Bogdan Walkowiak

33. **DOES SURFACE STRUCTURING OF METALLIC MATERIALS AFFECT THROMBOCOMPATIBILITY?**
Marta Kamińska, Marta Walczyńska, Magdalena Walkowiak-Przybyło, Piotr Komorowski, Bogdan Walkowiak

34. **DETERMINATION OF *ALOE VERA* RELEASE FROM ALGINATE BASED HYDROGELS**
Małgorzata Miastkowska, Katarzyna Bialik-Wąs

35. **STUDIES ON THE ENCAPSULATION OF MODEL ACTIVE SUBSTANCE USING THERMOSENSITIVE POLYMERIC NANOCARRIERS**
Katarzyna Bialik-Wąs, Paulina Sapuła, Krzysztof Pielichowski

36. **UNDERSTANDING OF FLUOROURACIL NANOPARTICLES SONOCHEMICAL FORMATION FOR ITS CONTROLLED DELIVERY FROM POLYMERIC SURFACES**
Paulina Chytrosz, Monika Gołda-Cępa, Lukasz Cwilklik, Waldemar Kulig, Andrzej Kotarba

37. **FUNCTIONALIZATION OF POLYURETHANE SURFACES FOR IMPROVED BIOCOMPATIBILITY**
Paulina Chytrosz, Monika Gołda-Cępa, Andrzej Kotarba

38. **SCIENTIFIC FUNDAMENTALS FOR ANTIBACTERIAL SURFACES DESIGN AND PREPARATION**
Wojciech Pajerski, Joanna Duch, Monika Golda-Cepa, Dorota Ochonska, Iwona Szymanska, Witold Reczynski, Monika Brzychczy-Wloch, Andrzej Kotarba

39. **BACTERIA MEET NANOPARTICLES - ELECTRON MICROSCOPY INSIGHTS**
Wojciech Pajerski, Monika Golda-Cepa, Miroslawa Pawlyta, Magdalena Parlinska-Wojtan, Monika Brzychczy-Wloch, Andrzej Kotarba

40. **HOW TO STIMULATE POLYMERIC SURFACES BIOCOMPATIBILITY AND HYDROXYAPATITE FORMATION: EXPERIMENTS SUPPORTED BY MD SIMULATIONS**
Monika Golda-Cepa, Paulina Chytrosz, Kamila Riedlova, Waldemar Kulig, Lukasz Cwiklik, Andrzej Kotarba

41. **DESIGN AND TAILORING OF POLYMERIC BIOMATERIALS SURFACES: PREVENTION OF BIOMATERIALS-CENTERED INFECTIONS**
Monika Golda-Cepa, Paulina Chytrosz, Wojciech Pajerski, Monika Brzychczy-Włoch, Andrzej Kotarba

42. **MODIFICATION OF CARBON MATERIALS SURFACES BY PLASMA: EFFECT OF THE INTRODUCED FUNCTIONAL GROUPS ON BIOCOMPATIBILITY AND ADHESION OF MICROORGANISMS**
Joanna Duch, Wojciech Pajerski, Monika Golda-Cepa, Dorota Ochonska, Witold Piskorz, Monika Brzychczy-Wloch, Jakub Rysz, Andrzej Kotarba

43. **CHARACTERIZATION OF PARTIALLY COVERED SELF-EXPANDABLE METALLIC STENTS FOR ESOPHAGEAL CANCER TREATMENT: IN VIVO DEGRADATION**
Paulina Chytrosz, Monika Golda-Cepa, Janusz Wlodarczyk, Jaroslaw Kuzdzal, Miroslawa El Fray, Andrzej Kotarba

44. **CHITOSAN HYDROGELS MODIFIED WITH SILK, GRAPHENE OXIDE OR REDUCED GRAPHENE OXIDE**
Barbara Szaraniec, Marcin Boruchowski, Karol Gryń, Jan Chłopek

NOVEL NANOCOMPUTED TOMOGRAPHY (nanoCT) TECHNIQUES APPLIED TO DENTAL RESEARCH

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[ENGINEERING OF BIOMATERIALS 158 (2020) 5]

Introduction

X-ray computed tomography (CT) has developed rapidly over the last decade, and the technological advances and user friendliness have made the technique applicable to virtually all branches of the natural sciences and manufacturing industries. The unique advantages of X-ray imaging reside in the combination of high resolution, the penetrating ability of looking inside materials even through complex sample environments, and the relative ease with which the resulting images can be interpreted. Still, X-ray imaging keeps progressing and near-future breakthroughs will include ultrafast measurements, computer-assisted image interpretation and lower-dose measurements. 3D images are easy to grasp, and CT is quite literally an eye-opener to internal processes in living and functional objects. With demand for higher resolutions in laboratory-based X-ray CT imaging in the submicrometer range, the fields of microcomputed tomography (microCT) and nanocomputed tomography (nanoCT) imaging have been established (within this work microCT refers to voxel sizes >1 µm and nanoCT refers to voxel sizes from >100 nm to <1 µm). Also for the general public and popular dissemination, CT images are easily digested and of invaluable importance when it comes to visualizing complex internal structures. CT has become an invaluable tool for the nondestructive investigation of objects in many fields such as material science, industrial testing, or medical diagnostics.
Technically, a range of different contrast mechanisms can be employed (diffraction, scattering, and absorption) where the spatial resolution may reach a few nanometres, while temporal resolutions can be below one microsecond. To become almost comparable to conventional histology with regard to resulting contrast, nanoCT imaging makes use very small focal spot sizes. The application of microCT and nanoCT for biological sample screening, however, remains limited due to very low intrinsic contrast of soft tissue, which means that soft tissues does not appear on regular X-ray. However, the utilization of different contrast agents can aid in visualize such soft tissue structures. Here we will present some recent advances in X-ray enhancement agents used on teeth and hydrogels, which has enabled us to visualize and quantify both soft and cellular tissues. The presentation will show that with our protocols it is possible to visualize cells alongside with mineralised tissues.to minimal speculation about the significance of the work.

Methodology

PTA in various concentrations and immersion time was tested and scanned with high resolution nano-CT. The method was applied to freshly extracted teeth where we examined both cementum and pulpal region.

Results

Three-dimensional nano-CT imaging of dental cementum and periodontium as well as interior components, such as odontoblasts and predentine, with high resolutions was made visible when using PTA staining. The optimal staining protocol differed in different segment of the tooth. The thickness of the cementum could be computed over the height of the tooth made possible by the PTA-enhanced contrast, and the attached soft tissue components of the interior of the tooth could be shown on the dentine-pulp interface in great detail. Three-dimensional illustrations allowed a histology-like visualization of the sections in all orientations with a single scan and easy sample preparation. Furthermore, the dentinal tubules, with the characteristic sigmoid curvature, could be visualized. The segmentation of the tubules and the surrounding dentine allowed a three-dimensional investigation of the dentine composition, such as tubular lumen or ratio of tubular lumen area to dentinal surface

Conclusion

The developed methodology show that it is possible to visualise hard tissue along with cellular structure and soft tissues using laboratory based nano-CT technique. The staining protocol depended on both tissue type and size. The methodology offers new possibilities for the visualisation of structures at the interphases between soft and hard dental tissue, particularly related to endodontic and periodontal research.

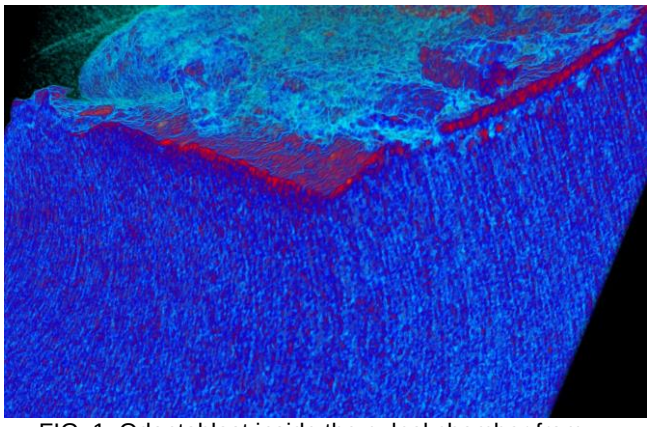

FIG. 1. Odontoblast inside the pulpal chamber from a freshly extracted tooth.

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MATERIAL ENGINEERING IN REGENERATIVE CARDIAC SURGERY: "YESTERDAY AND TODAY" & THE ROLE OF MATERIAL ENGINEERING IN THE RECONSTRUCTION OF DEFECTS IN ONCOLOGICAL DISEASES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 6]

Introduction

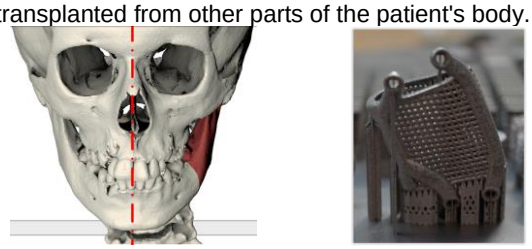
The first issue currently being developed is the topic of biological valves. It is a continuation of the idea proposed by Professor Zbigniew Religa, according to which based on the natural material of the extracellular matrix, bioactive valve prostheses are created. The valve scaffold is an acellular, xenogeneous or allogeneous tissue on which autologous cells are anodized in vitro or in vivo. Such a valve should have a potential for growth, self-repair and remodeling, similar to native tissue. A bioprosthesis of this kind should significantly bypass the limitations of commercial valve prostheses. The second issue, which is currently being implemented by the consortium partners in an international (Polish-Austrian) research and development project, and whose continuation is planned as part of the implementation project, concerns the mechanical valve for use in heart support chambers (FIG. 1).

a.) b.)

FIG. 1. Technology readiness level of the heart assist system chamber a.) new valve prototype b.) chamber with implemented chamber.

The main objective is to improve the effectiveness of treatment of patients with myocardial insufficiency, using heart support systems, by developing an innovative material solution consisting in the preparation of a composite material for contact with blood, understood as a combination of a metallic frame with a biocompatible layer in a polymer sheath, which will enable redesigning mechanical heart valves in ReligaHeart EXT (clinically applied VAD) and providing solutions for use in a pediatric blood pump (ReligaHeart PED). The results of the project are of great importance in terms of material, technological and biomedical aspects from the point of view of very active research and development work in Poland on the regeneration of the cardiovascular system developed within the Polish Cardiac Assist System - ReligaHeart. The commercial benefits will result from the possibility of production by Polish Small and Medium Enterprises (SMEs), which have 30 years of successes on the Eastern European market.

Another issue in the field of cardiac surgery is the development of fully hemocompatible blood pump rotors. The innovative ReligaHeart ROT rotary implantable blood pump was developed in the Foundation for Cardiac Surgery Development, a close associate of the consortium members. The chamber is in the preclinical research phase in patients with advanced myocardial dysfunction. It is a mechanical bearingless pump, equipped with a rotor suspended magnetically and hydraulically, which provides a flow of up to 10 l/min at 30-45% capacity. A fully magnetic rotor suspension system, without hydrodynamic bearings, is being developed to reduce shear stress on the blood and protect Von-Willebrand platelets and proteins from damage causing the risk of bleeding. ReligaHeart VASC, is currently under development and is designed for short-term cardiac support in cardiac shock. It has an implantable rotor system with a miniaturized motor and magnetic rotor suspension system. Biomaterial engineering works on the reconstruction of the cranial cavity in case of necessity of skeleton resection of cranial parts. In general, such jaw and mandible resections are difficult and leave large bone losses in patients' faces. The advantage of the new materials is the possibility of local release of radiopharmaceuticals and the safety for high doses of radiotherapy without the risk of necrosis. In the case of benign and malignant tumors affecting the maxillofacial region, jaw bone resection reflects standard therapy (FIG. 2). The resulting large bone losses lead to scars, facial malformations, chewing loss and probably speech. In the next stage, plastic surgery is necessary to restore the correct physical and physiological properties. Reconstruction of the removed parts of the skull is also necessary from a psychological point of view. Although vascularized bone autographs reflect the current gold standard in this type of therapy, only a small part of the bones in the patient's body is available for transplantation. Our research has focused on creating alternative treatment techniques: tissue reconstruction with an innovative mandibular implant that stimulates bone tissue growth. This solution has found particular interest among oncological centers dealing with facial reconstruction, such as Oncology Center in Gliwice. On the basis of the results obtained so far, further development of the issue is planned within the framework of subsequent projects, based on specific clinical cases. A group of about 20 patients with benign tumors (dentiac disease, enamel, cyst, giant cell tumor) around the mandibular body or branches will be investigated. The main aim of the research in this area will be to assess the effectiveness of the use of artificial materials for the reconstruction of the mandible in order to replace methods of using free bone lobes vascularized, transplanted from other parts of the patient's body.



a.) b.)

FIG. 2. Jaw implant prototype prepared individually for the patient.

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CURRENT STATUS AND FUTURE PROSPECTS OF GENOME-SCALE METABOLIC MODELING TO OPTIMIZE THE USE OF MESENCHYMAL STEM CELLS IN REGENERATIVE MEDICINE AND BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 7]

Abstract
Mesenchymal stem cells are a promising source for externally grown tissue replacements and patient-specific immunomodulatory treatments. This promise has not yet been fulfilled in part due to production scaling issues and the need to maintain the correct phenotype after re-implantation. One aspect of extracorporeal growth that may be manipulated to optimize cell growth and differentiation is metabolism. The metabolism of MSCs changes during and in response to differentiation and immunomodulatory changes. MSC metabolism may be linked to functional differences but how this occurs and influences MSC function remains unclear. Understanding how MSC metabolism relates to cell function is however important as metabolite availability and environmental circumstances in the body may affect the success of implantation. Genome-scale constraint based metabolic modelling can be used as a tool to fill gaps in knowledge of MSC metabolism, acting as a framework to integrate and understand various data types (e.g., genomic, transcriptomic and metabolomic). These approaches have long been used to optimize the growth and productivity of bacterial production systems and are being increasingly used to provide insights into human health research. Production of tissue for implantation using MSCs requires both optimized production of cell mass and the understanding of the patient and phenotype specific metabolic situation. This review considers the current knowledge of MSC metabolism and how it may be optimized along with the current and future uses of genome scale constraint based metabolic modelling to further this aim.

NEW COMPOSITES BASED ON BACTERIAL NANOCELLULOSE AND GELATIN

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[ENGINEERING OF BIOMATERIALS 158 (2020) 8]

Introduction

Gelatin is a biopolymer which derives from collagen. Gelatin itself is widely applied in the food industry and for preparation of biomedical and cosmetic materials.
Bacterial nanocellulose (BNC), also defined as microbial cellulose, is produced by bacteria. The valuable properties of BNC include, in particular, unique biological, physicochemical and mechanical properties. An example of exceptional properties are high crystallinity, high water-holding capacity, excellent tensile strength and also Young's modulus. BC is used in medicine as wound dressings, drug carriers, medical implants and in cosmetology. It also has a significant role in various industries, i.e. food, paper, textile, chemical industries. Gelatin and BNC have been widely used for production of 3D sponges, wound dressings and scaffolds for biomedical applications [1]. The blends of these biopolymers with other compounds are also widely used in cosmetic preparations [2]. Binary blends and composites of two natural polymers can lead to preparation of new materials suitable for biomedical applications [1,3]. In this work, the composites based on bacterial nanocellulose and gelatin were prepared and its properties were studied.

Materials and Methods

Bacterial nanocellulose was obtained from the Center of Polymer System Tomas Bata University in Zlin, Czech Republic. Gelatin was purchased by Sigma-Aldrich company.
Bacterial nanocellulose was blended with gelatin and the composite was obtained. The structure of the composites was evaluated by attenuated total reflection infrared spectroscopy and Scanning Electron Microscope (SEM) pictures. Surface properties of thin films were analyzed by AFM and contact angle measurements. Swelling properties were also studied. Preliminary biological test has been done.

Results and Discussion

Blending of BNC and gelatine led to the porous composite. IR spectroscopy showed that between components of the composite there are interactions, mainly due to hydrogen bond. Example of FTIR spectrum is shown in FIG. 1. According the structure of single biopolymers the interactions are due to hydrogen bonds formed between chemical moieties of polymers. SEM image of the composite is shown in FIG. 2.

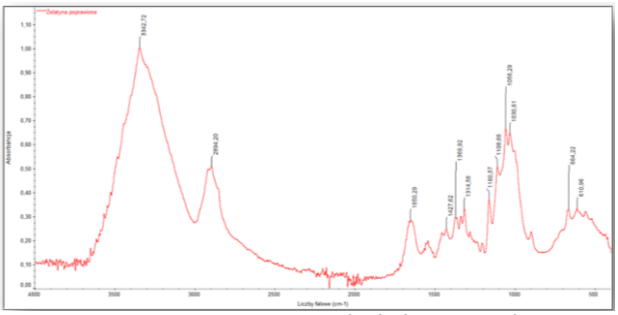

FIG. 1. FTIR spectra BNC/gelatin composite.

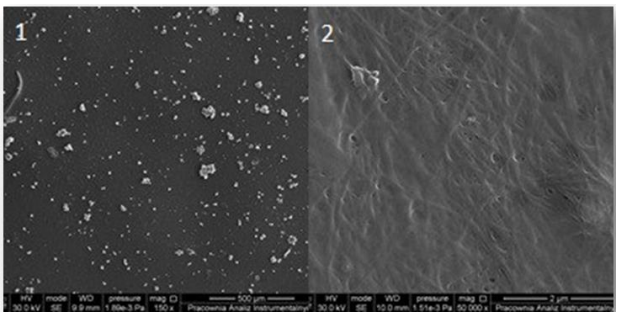

FIG. 2. SEM image of BNC/gelatin composite: 1) 500 µm, 2) 2 µm.

New composite based on BNC and gelatin shows good swelling properties. Preliminary biological studies showed that the material obtained is biocompatible.

Conclusions

Strong interactions between bacterial nanocellulose and gelatin lead to the new composite material. The swelling properties of new material can be useful in biomedical and cosmetic applications. New material can be considered as wound dressing material.

Acknowledgments

Authors acknowledge Center of Polymer System Tomas Bata University in Zlin for preparation of bacterial nanocellulose for this study and COST Action ENBA for support the research stay of Oliwia Mężykowska at Tomas Bata University in Zlin.

References

[1] A. Sionkowska, Prog. Polym. Sci. 36 (2011) 1254-1276.
[2] A. Sionkowska, B. Kaczmarek et. al., Pure Appl. Chem. 89 (2017) 1829-1835.
[3] B. Kaczmarek, A. Sionkowska, Int. J. Biol. Macromol. 107 (2018) 470-477.

RHEOLOGICAL PROPERTIES OF COLLAGEN GELS AFTER UV TREATMENT

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[ENGINEERING OF BIOMATERIALS 158 (2020) 9]

Introduction

Fish collagen is commonly applied in food and biomaterials production and can replace mammalian collagen; however, low denaturation temperature is usually its main disadvantage. Collagen extracted from the *Silver Carp* skin has attracted much attention in recent years due to its relatively high denaturation temperature [1]. As far as collagen in a solution is concerned, several properties can be measured. Rheological characteristics of collagen solutions and gels at different concentrations is one of them. Rheological properties of collagen are essential in several applications. In the case of cosmetic, biomedical and food applications, its rheological behaviour at different temperature values is essential in order to design a proper formulation and check its applicability under several conditions. Collagen gels are also used for studying cell–matrix mechanical interactions as well as developing tissue equivalents, the rheological properties of which are very important, too [2]. For several applications, collagen gels need to be sterilized and for this purpose the UV radiation can be used.
The aim of this work was to study the rheological behaviour of collagen obtained from the *Silver Carp* skin and the influence of UV irradiation on rheological properties of collagen gels.

Materials and Methods

Collagen was purchased from WellU sp. z.o.o, Gdynia, Poland. It was obtained by collagen isolation from the *Silver Carp* skin. The skin fragments were removed manually and washed with chilled tap water to get rid of the adhering tissues. In the next stage, the material was disinfected with 3% hydrogen peroxide water solution, residues of which were further rinsed off. The purified skin was placed in a lactic acid solution and left for 3 days to extract the collagenous proteins.
The collagen solution was dialyzed against distilled water for 2 days and then lyophilized. After lyophilisation, collagen gels were prepared in diluted 0.1M acetic acid at the concentrations of 5 mg/mL and 10 mg/mL. For prepared collagen gels, the rheological properties were measured. Collagen gels were irradiated with UV light and again the rheological properties were measured.
A rheological investigation was carried out on the prepared samples by means of a rotational viscometer, Bohlin Visco 88 (UK), equipped with a heating system and a solvent trap kit. Collagen solutions were irradiated using UV lamp ULTRAVIOL NBV 15, which emits mainly UVC with 254 nm wavelength. Collagen solutions were irradiated in a distance of 5 cm from the UV lamp.

Results and Discussion

FIG. 1 reports the viscosity curves of collagen solutions with concentration 5 mg/mL before and after UV irradiation with wavelength 254 nm.

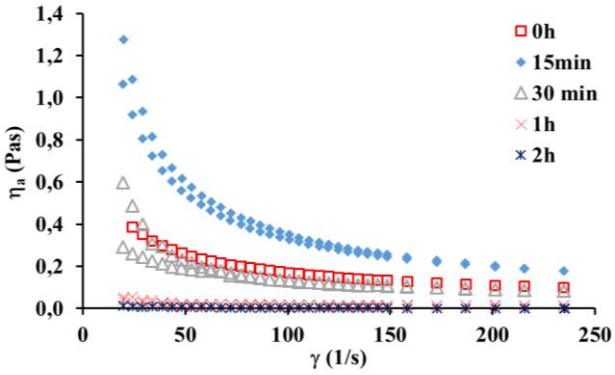

FIG. 1. Viscosity curves for 5 mg/mL collagen solution at 20°C before and after various time of UV-irradiation.

A collagen solution is characterized by the typical shear-thinning behaviour of polymer solutions observed in the decrease in the viscosity as the shear rate increases due to the progressive orientation and disentanglement of the chains. The apparent viscosity of the collagen solution is heightened with increasing concentrations. While the concentration of the collagen solution increases, the viscosity curves present a more pronounced shear-thinning behaviour. This is caused by an increase in collagen macromolecules interactions leading to the increase in the entanglement of the chains and more pronounced non-Newtonian behaviour.
After 15 min of UV irradiation an increase of viscosity was observed. Such an increase can be a result of physical crosslinking of collagen molecules by free radicals induced by UV light. However, after longer treatment than 15 min a decrease of viscosity was observed. After 1 hour of UV treatment collagen molecules lost its ability for gel formation. It may suggest that collagen molecules are fully denatured after 1 hour of UV treatment and collagen lost totally its native structure.

Conclusions

UV treatment of collagen gels obtained from the *Silver Carp* fish skin leads to the crosslinking reaction after short time of UV irradiation. After 1 hour of UV treatment collagen was fully denatured and lost its ability for gel formation.

Acknowledgements

Authors acknowledge WellU company for preparation of collagen for this study and COST Action ENBA for support the research stay of students at Tomas Bata University in Zlin.

References

[1] Zhang, J., Duan, R., Tian, Y., & Konno, K. (2009) Characterisation of acid-soluble collagen from skin of silver carp (Hypophthalmichthys molitrix). Food Chemistry 116, 318–322.
[2] Knapp, VD.M., Barocas, V.H., Moon, A.G., & Robert T Tranquillo, R.T. (1997) Rheology of reconstituted type I collagen gel in confined compression. Journal of Rheology 415(5).

THE EFFECT OF β-GLUCAN ON THE SURFACE MORPHOLOGY AND ROUGHNESS PARAMETERS OF FISH ORIGIN COLLAGEN MATERIALS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 10]

Introduction

The main sources of collagen for industrial applications are pork and beef skins and bones [1]. However, their application has been restricted because of the possibility to transfer some animal diseases (e.g. foot and mouth disease, bovine spongiform encephalopathy) to humans [2]. In connection with the above, more and more popular become fish collagen. Fish origin collagen may be extracted from fish skin, scale, spines, dorsal strings, and swim bladders. Therefore, this protein extracted from fish by-products has a great interest in the cosmetics, pharmaceutical, and medical industries. Using by-products from the local fish industry may decrease collagen cost and allows it to mitigate environmental problems [3]. However, the properties of collagen may not full fill all requirements of materials for medical use. Therefore, to improve the properties of collagen material β-glucan was added. It is known that β-glucan may stimulate wound healing. In addition to improving the biological properties of the material is able to form a crosslinking network by hydrogen bonds and improve physicochemical properties [4]. The aim of this work was to evaluate the influence of β-glucan addition on the surface morphology and roughness parameters of collagen materials.

Materials and Methods

Collagen was obtained in our laboratory from *Aristichthys nobilis* skin. 1% (w/v) collagen solution was prepared with 0.1M acetic acid as a solvent. 5% β-glucan was prepared in deionized water and heat in a water bath without boiling. Solutions were mixed together in collagen/ β-glucan ratio: 100/0; 90/10; 70/30; 50/50 (w/w). Materials were obtained by the solvent casting method.
The topographic structure of the polymeric film was observed using an atomic force microscope. Images were obtained using a multimode scanning probe microscope with a Nanoscope IIIa controller (Digital Instruments, Santa Barbara, CA) operating in the tapping mode, in air, at room temperature.

Results and Discussion

AFM picture of Coll70/30BG material is presented on FIG. 1. and complementary data about all roughness parameter are presented in TABLE 1. Can be seen, that prepared material was compact and non-porous. On surface of Coll70/30BG one can see microfolds formation. Rq and Ra parameters increases with incising amount of β-glucan into materials. Both parameters increase almost 4 times for Coll50/50BG compared to pure collagen. The average height of material with 50% β-glucan content amounted 100 nm.

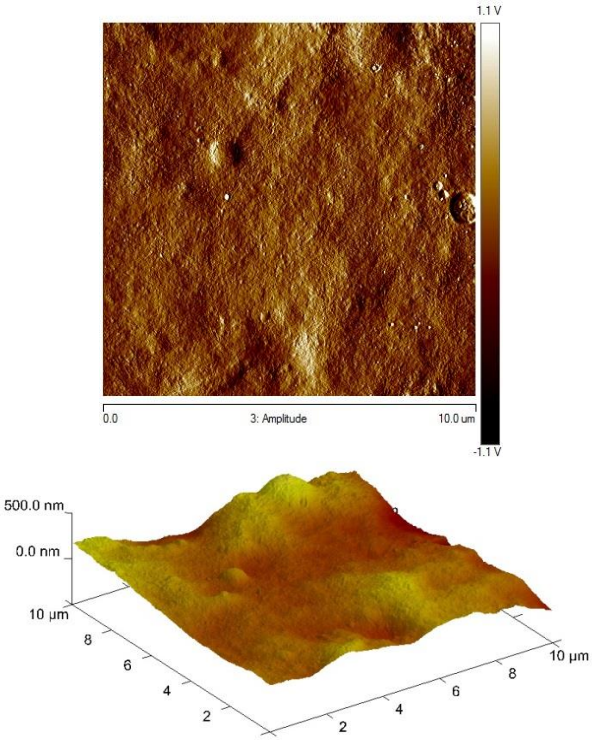

FIG. 1. Example pictures of structure morphology of Coll70/30BG films.

TABLE 1. Surface roughness parameters for different kinds of films.

Specimen	Rq [nm]	Ra [nm]
Coll	33.4	26.2
Coll90/10BG	36.5	29.2
Coll70/30BG	59.0	47.8
Coll50/50BG	125	100

A film composed of β-glucan from Sacharomyces cerevisiae origin was a non-porous and characterized granular-like structure with 100 nm high [5]. The thermal method used for prepared β-glucan films resulted in a smooth surface. However, materials prepared by the dialysis method were characterized by randomly distribute precipitates [6].

Conclusions

Fish skin from Aristichthys nobilis may be used as collagen source and material based on this protein and β-glucan may be obtained by the solvent evaporation method. The addition of β-glucan to collagen material increases the surface roughness without visible precipitates. The method used for materials preparation influence on the surface structure.

Acknowledgments

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References

[1] G.K.S. Arumugam, D. Sharma *et al*., Sustain. Chem. Pharm., 9 (2018) 19-26.
[2] R. Ahmed, A Tilahun Getachew *et al*., LWT 89 (2018) 44-51.
[3] S.F Bruno, F. Jr. Anta Akouan Ekorong *et al*., Trends Food Sci Tech., 85 (2019) 10-22
[4] L.I. Jesus, R. Fhernanda *et al*., Int. J. Biol. Macromol.,117 (2018) 1361-1366.
[5] M. Novák, A. Synytsya *et al*. Carbohydr. Polym., 87 (2012) 2496-2504.
[6] K. Klimek, A. Przekora *et al*. Carbohydr. Polym.,164 (2017) 170–178.

CHITOSAN DERIVATIVES - SYNTHESIS AND CHARACTERIZATION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 11]

Introduction

Recently, interest in chitin, chitosan and its derivatives, in particular carboxymethyl chitosan, has increased. It is associated not only with the growing interest in environmental protection or ecology but with their properties. Chitin belongs to the group of biodegradable polymers. It is a polymer that occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthro-pods or in the cell walls of fungi and yeast. It is also produced by several other living organisms in the lower plant and animal kingdoms. The most important derivative of chitin is chitosan. When the degree of deacetylation of chitin reach about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. Chitosan is biocompatible, biodegradable, non-toxic and low immunogenicity. Unfortunately, it has low solubility in water. This problem is solved by its water-soluble derivatives such as carboxymethyl chitosan. This is polymer in which the carboxymethyl group is introduced at various places either into the amino group or into the hydroxyl group on the C6 atom. It is used in medicine as a wound dressing, drug delivery or used in tissue engineering. Carboxymethyl chitosan is a polymer that has better biological and physicochemical properties compared to chitosan. [1-4]

Materials and Methods

Chitosan, low molecular weight was supplied by Aldrich Chemical Company, Inc. Sodium hydroxide, sodium chloride was purchased from POCH S.A. (Avantor, Poland). Chloroacetic acid Hydrochloric acid, Isopropyl alcohol was supplied Chempur (Poland). The methods of synthesis were taken from the literature (C. Yu et al. and A. Labidi, et al.).

2.7 g of sodium hydroxide was dissolved in 4 mL of H_2O and 16 mL of isopropanol and mixed with 2 g of chitosan. The solution was left at room temperature for 1 h. Next, 3 g of monochloroacetic acid in 4 mL of isopropanol were dropwise added to the mixture for 30 min. Later mixture was in under reflux at 55°C for 4 h. After cooling, impurities were removed by filtration. The product was precipitated by the addition of 80% ethanol. The product was then dried under vacuum or lyophilized.

2 g chitosan was added into 7.6 g 40% NaOH aqueous solution. Then, 20 mL isopropyl alcohol with 3.4 g chloroacetic acid were added into the flask Afterwards mixture was in under reflux at 65 °C for 4 h. After cooling, impurities were removed by filtration. The product was precipitated by the addition of 80% ethanol. The product was then dried under vacuum or lyophilized. The obtained chitosan derivatives were characterized using capillary viscometer and spectroscopy study.

Results and Discussion

In ethanol, chitosan derivatives formed white fibrous structures. After drying, the synthesis products had a slightly yellow colour. Depending on the synthesis, the intensity of the colour varied. Based on the spectroscopic analyses, one can notice differences between the obtained chitosan derivatives. The viscosity of carboxymethyl chitosan solutions and the average molecular weight were determined based on viscometric measurements. The results obtained for individual derivatives are different.

Conclusions

Analysis of the results shows that the change of synthesis parameters affects to the properties of the chitosan derivatives.

Acknowledgements

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References

[1] Chen Yu, Liu Yun-fei, et al., Carbohydr. Polym. 75, (2009) 287-292.
[2] A. Labidia, A. Salaberria, et al., Microchem. J. 148 (2019) 531–540.
[3] K. Lewandowska, Int. J. Biol. Macromol. 147 (2020) 1156–1163.
[4] N. Kahya, Polym. Sci. 5 (2019) 1-11.

CHARACTERIZATION OF THIN CHITOSAN COMPOSITE FILMS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 12]

Introduction

Chitosan is a derivative of chitin obtained most often from crustacean shells although it is also produced by other organisms, e.g. Mushrooms. Chitin is a substance that is hard to dissolve. In contrast, chitosan can be dissolved in dilute acids, such as lactic acid or acetic acid, which do not harm humans and the environment. Chitosan is used in the cosmetics, pharmaceutical and medicine industries. To discover its potential, various substances are added to chitosan, such as ionic liquids, inorganic additives, other polymers. The aim of the study was to determine the effect of addition of nanoclay on the properties of the composite in the form of thin films. Thin films have been formed by casting methods from lactic acid solution. Morphology and swelling behavior of chitosan composites before and after neutralization process were studied.

Materials and Methods

Chitosan was supplied by Institute of Sea Fisher (Poland). Nanoclay was supplied by Aldrich Chemical Company. Lactic acid, sodium hydroxide and the substances needed to prepare phosphate buffered saline (PBS) were provided by POCh (Avantor) or Chempur Poland. All chemicals were of analytical grade and used as received without further purification.

To create thin films, 2.0 wt.% aqueous solution was prepared by dissolving chitosan sample in a lactic acid (0.1M). The additive of nanoclay (3% and 5% on solid chitosan) was dispersed in the solvent. Then, polymer solution was added slowly to the nano-additive dispersion. Polymer and composite films were achieved by a solution casting methods. Thin films were also neutralized by immersing into a sodium hydroxide solution (1%) for 15 min. After the alkaline treatment, the films were rinsed and left in distilled water, overnight.

Morphology of composite films were observed with SEM (Quanta 3D FEG, D9399, FEI Company, Eindhoven, the Netherlands).

Each type of film (10 mm x 10 mm) was immersed in phosphate buffered saline (PBS, pH = 7.4) solution. After 1, 4, 24 and 48 h, samples were removed from the PBS solution, were gently dried by putting them between two sheets of paper and weighted.

Results and Discussion

Unmodified chitosan film shows a relatively smooth flat surface morphology. For chitosan composites, the surfaces of films exhibited a few asperities, corresponding to the presence of nanoclay particles, which were homogeneously distributed.

The swelling test of the resulting composites without neutralization showed that the samples without neutralization dissolve in PBS. For neutralized samples, swelling results were different depending on the average molecular weight of chitosan and the concentration of nanoclay.

Conclusions

The morphology and swelling behavior of chitosan/nanoclay composite as thin films are affected by nanoclay concentration, average molecular weight of chitosan, and the neutralization process.

References

[1] P. S. Bakshi, D. Selvakumar et al., Int. J. Biol. Macromol. 150 (2020) 1072-1083.
[2] K. Lewandowska, Int. J. Biol. Macromol. 147 (2020) 1156–1163.
[3] M. C.G. Pellá, M.K. Lima-Tenório et al., Carbohydr. Polym. 196 (2018) 233-245.

SURFACE-MODIFIED SPION SYSTEMS FOR CANCER THERAPY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 13]

Introduction

About 90% of all cancer-related deaths are due to metastasis – a process in which circulating tumor cells (CTC) detach from the primary tumor site, and through the bloodstream travel to the other, sometimes distant organs, where they form secondary tumor. Epithelial-mesenchymal transition (EMT) enables cancercells to suppress their epithelial features and activate mesenchymal ones, allowing them to migrate from the primary tumor as CTC. As a result of EMT process E-cadherin on the surface of cancer cells is replaced by N-cadherin. Anti-N-cadherin antibodies can thus be used to effectively target CTC. Superparamagnetic iron oxide nanoparticles (SPION) exhibit a number of unique properties, which make them a subject of growing interest of scientific community. SPION are small crystals of iron oxide, usually magnetite (Fe_3O_4) or its oxidised form, maghemite (γ-Fe_2O_3). Many properties of SPION can be controlled by their coating, as well as by a further modification of their surface.

Our aim was to obtain SPION stabilized by cationic derivative of chitosan and decorated with anti N-cadherin antibodies, which could be used as a targeting system able to selectively bind to CTC. We have also planned to introduce methotrexate to the surface of SPION. Such systems may be used either to deliver an anticancer drug (methotrexate) or to magnetically capture CTC - either for diagnostic or therapeutic purposes.

Materials and Methods

The cationic derivative of chitosan (CCh) was obtained in the reaction between chitosan and GTMAC (glicydyltrimethylammonium chloride). SPION stabilized with CCh (SPION/CCh) were obtained *via* co-precipitation of Fe^{2+} and Fe^{3+} salts with ammonia in the presence of CCh. Magnetic chromatography was used to purify the nanoparticles. SPION were then tosylated. in reaction with p-toluenesulfonyl chloride. Tosylated SPION/CCh (SPION/CCh-Tos) were reacted with anti-N-cadherin antibodies in borate buffer (pH = 9.5) in the presence of ammonium sulfate at 37°C [1]. Methotrexate (MTX) was attached to SPION/CCh using EDC/NHS chemistry.

CCh was characterized using ^{1}H NMR, ATR-FTIR and elemental analysis. SPION/CCh were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and zeta potential measurements. Tosylation was confirmed using ATR-FTIR. MTX attachment was verified using UV-Vis absorption spectroscopy. The success of the antibodies attachment to SPION/CCh was confirmed using immunostaining with fluorescent secondary antibodies (NorthernLights™ anti-sheep IgG-NL557)

Human prostate cell lines (American Type Culture Collection): LNCaP (androgen-dependent cell line derived from lymph nodes metastasis) and PC-3 (androgen independent cell line derived from bone metastasis) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. MTT test was used to evaluate the cytotoxicity of SPION modified with anti-N-cadherin antibodies and MTX. The unbinding force AFM measurements were performed using atomic force microscope equipped with a ''liquid cell'' setup, in culture medium, at room temperature. Cells lysis and Western Blot were carried out as previously described [2-4]. Confocal microscope was used to visualize the interaction between cells and specific antibodies or SPION/CCh with bound anti-N-cadherin antibodies (SPION/CCh-N-cad). SPION/CCh-N-cad were also stained with secondary fluorescent antibodies, incubated with CTC and studied by flow cytometry.

Magnetic properties of various SPION systems, were determined using Vibrating Sample Magnetometer. 57Fe Moessbauer measurements were carried out in the transmission mode at a constant acceleration spectrometer with 50 mCi 57Co/Rh source.

Results and Discussion

CCh was successfully synthetized and used to obtain SPION/CCh. The average size of the obtained nanoparticles was 143 ± 21 nm, and their zeta potential was high (37.7 ±1.8 mV), confirming they were colloidally stable. The surface of SPION/CCh was successfully decorated with anti-N-cadherin antibodies and MTX. Magnetic studies confirmed superparamagnetic character of the studied SPION systems. Confocal microscopy revealed that SPION/CCh are effectively taken up by cancer cells. MTT assay showed that SPION/CCh system is cytotoxic to PC-3 prostate cancer cells after 24 h of incubation. Western Blot analysis gave an insight in the differences in protein expressions in the untreated cells and cells exposed to SPION/CCh. Flow cytometry studies and AFM analysis allowed to confirm the specific binding of the targeted system to PC-3 prostate cancer cells. Preliminary studies showed effective magnetic capture of cancer cells with attached SPION/CCh-N-cad.

Conclusions

We have synthetized SPION nanoparticles stabilized with CCh, and successfully decorated their surface with anti-N-cadherin antibodies and MTX. The physicochemical, biological and magnetic properties of the obtained systems were studied. Preliminary studies on the SPION/CCh-MTX and SPION/CCh-N-Cad systems confirmed their potential in targeted therapy of cancer.

Acknowledgments

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References

[1] A. Kaczyńska, K. Guzdek-Zając et al., RSC Adv. 6 (2016) 72586-72595.
[2] J. Dulińska-Litewka, J.A. McCubrey *et al*., Curr. Med. Chem. 20 (2013) 144-157.
[3] E. Górowska-Wójtowicz, A. Hejmej *et al.,* Toxicol. In Vitro 40 (2017) 324-335.
[4] D. Gil, M. Zarzycka *et al*., Hum. Cell 32 (2019) 379-389.

HALLOYSITE-BASED SYSTEM FOR CONTROLLED DELIVERY OF CLINDAMYCIN PHOSPHATE

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[ENGINEERING OF BIOMATERIALS 158 (2020) 14]

Introduction

Halloysite (HNT) is a two-layered (1:1) aluminosilicate, chemically similar to kaolinite. It exhibits a range of morphologies, among which the predominant form is a hollow tubular structure of the submicrometer scale. HAL nanotubes are able to encapsulate low molecular weight drugs (e.g. antibiotics, anti-inflammatory agents) [1], which may be then slowly released to provide protection against inflammation or bacterial infection. The uniqueness of the HNT as a tubular carrier lies in the fact that, due to the opposite charges of its inner lumen (positive) and outer surface (negative), electrostatic interactions may be used to increase efficiency and selectivity of the entrapment of the bioactive agents.

Clindamycin is an antibiotic used to treat, among others, dental and skin infections. It is widely applied in implant sterilization and bone disease treatment, as it has high ability of penetration of the bone tissue [2]. Its positive role in the late stages of osteogenesis have been recently reported in the literature [3].

Our aim was to obtain a halloysite nanotubes based system for a controlled, prolonged delivery of clindamycin phosphate. Nanotubes were additionally etched to increase their loading ability. Such a system could be applied as a component of bone and skin regeneration materials (scaffolds, dental fillings, wound-dressings).

Materials and Methods

Halloysite nanotubes were pre-treated by etching with the 1 M acetic acid at 50°C, followed by extensive washing with water and freeze-drying. Clindamycin phosphate was dissolved in water (50 mg/ml) and then added either to the untreated (HNT) or to the etched (HNT-E) halloysite nanotubes and thoroughly mixed to obtain a stable suspension. Both nanoclays were then placed under reduced pressure (200 mmHg) to allow for the solution to be drown in into the nanotubes. The mixtures were left 15 minutes and then moved to the normal conditions to equilibrate. The cycle was repeated three times, then the sediments (HNT-Clin and HNT-E-Clin) were separated, washed with water and freeze-dried.

The morphology of the obtained nanoclay-drug systems was studied using ATR-FTIR, SEM, and TEM (FIG. 1). The release profiles for both nanoclays were studied in phosphate buffer saline (PBS) at pH = 7.4 and at 37°C. The concentration of clindamycin phosphate was measured throughout the release studies using Waters HPLC system with C18 column and UV-Vis detection. Acetonitrile:phosphate buffer (pH = 3) 20:80 mixture was used as an eluent. Encapsulation efficiency and loading of the antibiotic in the nanotubes of both untreated and etched HNT was established based on the amount of the drug released.

Results and Discussion

The SEM and TEM studies showed that the diameter of the inner lumen of the nanotubes was enlarged as a result of the etching process. SEM and ATR-FTIR studies confirmed that no unwanted processes, such as the degradation of the outer layer of the nanotubes, were observed. Clindamycin phosphate was successfully loaded into the HNT and HNT-E nanotubes. Within the first 24 h more drug was released from the HNT-E nanotubes than from untreated HNT. The long term release profile obtained for the HNT-E-Clin system showed that so-called “burst release” was observed, followed by a faster release for the first 4 hours and much slower but prolonged release of the antibiotic up to 2 days.

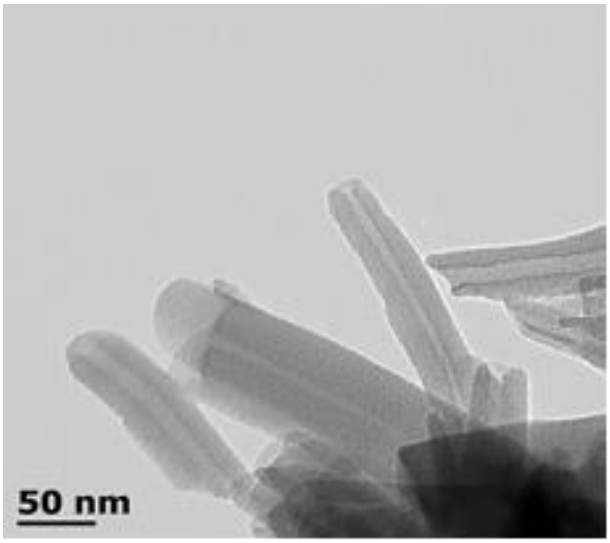

FIG. 1. TEM image of HNT-E nanotubes.

Conclusions

We have successfully loaded clindamycin phosphate into the halloysite due to the electrostatic interactions between a positively charged lumen of the nanotubes and negative charge of the drug. The proposed etching process allowed to increase the amount of drug released form the system. The obtained release profile was favourable for the proposed applications, as it provides an initial burst release of the antibiotic necessary to fight the infection, followed by e prolonged, slow release providing a long-term protection.

References

[1] R. Qi, R. Guo *et al.*, Colloids Surf B 110 (2013) 148-155.

[2] V. Zeller, A. Dzeing-Ella *et al.*, Antimicrob Agents Chemother 54(1) (2010) 88-92.

[3] M. Dalband, M. Isapour *et al.*, Regeneration, Reconstruction & Restoration 1(2) (2016) 124-127

BONE MORPHOGENETIC PROTEIN-2 ADSORPTION ON PHOTOACTIVE POLYMERIC SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 15]

Introduction

Incorporation of growth factors into the scaffold biomaterial can improve osteogenesis and angiogenesis. A number of bone-derived growth factors have been isolated and characterized from bone matrix, such as bone morphogenetic proteins (BMPs), which display mitogenic, differentiating, and osteolytic activities, allowing these molecules to act as potential determinants of local bone formation [1]. The main aim of this study was the effective covalent and electrostatic immobilization of BMP-2 onto polymeric multilayers (PEM).

Materials and Methods

Polymeric multilayers were composed from diazoresin (DR) and chondroitin sulphate (CHON). After adsorption, growth factors are immobilized onto PEM surfaces by electrostatic or covalent photochemical crosslinking [2]. Quartz crystal microbalance (QCM) was used to analyze the kinetics of structural and mass changes of BMP-2 adsorption on polymeric substrates. Atomic force microscopy (AFM) was used to verify morphology of the substrates. X-ray photoelectron spectrometer was used to analyze functional groups, exposed to bulk (angle resolved-XPS). Secondary-ion mass spectrometry (Tof-SIMS) gave us the information about protein spatial adsorption onto PEM multilayers.

Results and Discussion

The effective protein adsorption was confirmed by QCM. This technique gives an insight into the reaction kinetics as well as multilayers structure modification during adsorption. Much more BMPs were adsorbed on non-crosslinked multilayers. From the dissipate energy it was observed that the substrates became more stiff. AFM data showed that the proteins are efficiently deposited on the surface of the polymers. XPS measurements confirmed that all deposited polymeric layers penetrate each other. Hydroxyl and ester functional groups are exposed to the surfaces.

Conclusions

The result showed that the polymeric multilayers are effectively photocrosslinked. BMP-2 adsorb effectively on the PEM independently of the terminal layer and non-crosslinked and crosslinked layers. PEM films are the versatile substrate for proteins immobilization. Those research will determine the possibility of future usage of such PEM with BMPs as active coatings of implants or scaffolds enabling the proliferation and differentiation of stem cells.

Acknowledgments

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References

[1] S. Avnet, G. Di Pompo *et al.,* Cancer Metast. Rev. 38 (2019) 133-147
[2] D. W. Ham, E. C. Jang *et al.*, J. Ind. Eng. Chem. 40 (2016) 177-184

STEM CELLS RESPONSE ON PHOTO-CROSSLINKED HEPARIN – BONE MORPHOGENETIC PROTEINS (BMP-2/-7) HYBRID SYSTEMS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 16]

Introduction
Biomaterial scientists recognized the importance of the integrin-mediated signaling that influences key cellular responses, such as adhesion, migration, proliferation. The studies were focused on bone morphogenetic proteins (BMP-2 and BMP-7) embedment optimization onto polyelectrolyte multilayers (PEM). The covalent binding of two types of BMPs allow to decrease the required amount of proteins for efficient cell differentiation by creating the synergy pairs between them [1,2]. Than physicochemical and biological properties of such hybrid materials were examined as a function of stem cells response. The aim was to prepare active coatings of bone implants or scaffolds enabling the proliferation and differentiation of stem cells.

Materials and Methods
The studies were focused on bone morphogenetic proteins (BMP-2 and BMP-7) embedment optimization onto polyelectrolyte multilayers (PEM) composed from polyanion: heparin (HEP) and polycation: diazoresin (DR) as $(DR/HEP)_6$ system. PEM physicochemical properties before and after BMPs embedment, and before and after UV photo-crosslinking were optimized and analyzed by atomic force microscopy (AFM) and quartz crystal microbalance (QCM-D). MSCs biological responses were examined by MTT, trypan blue assays, immunocytochemistry staining (FIG. 1), and qRT-PCR technique.

Results and Discussion
The effective both BMP types immobilization was confirmed by AFM and QCM measurements. It was proven that chosen polymeric films enhanced cell viability, proliferation and adhesion. Moreover, covalent embedment of both BMPs enhances cells differentiation into bones, compared to those embed separately.

Conclusions
The reason for using PEM is dictated by that it still serves as a versatile platform allowing the use of various substrates on which they can be deposited. Examined PEM/BMPs systems are versatile coatings that can be used to coat a wide range of scaffolds or implants. Embedding of two different bone morphogenetic proteins (BMPs) combining with glycosaminoglycan - heparin gave the synergistic effect. Thanks to the covalent binding of two BMPs types, their osteogenic properties are enhanced by the activation of several signaling pathways. Such a method will reduce the required amount of growth factors for efficient stem cells differentiation, and lower the cost, both in in vitro culture and bone tissue engineering.

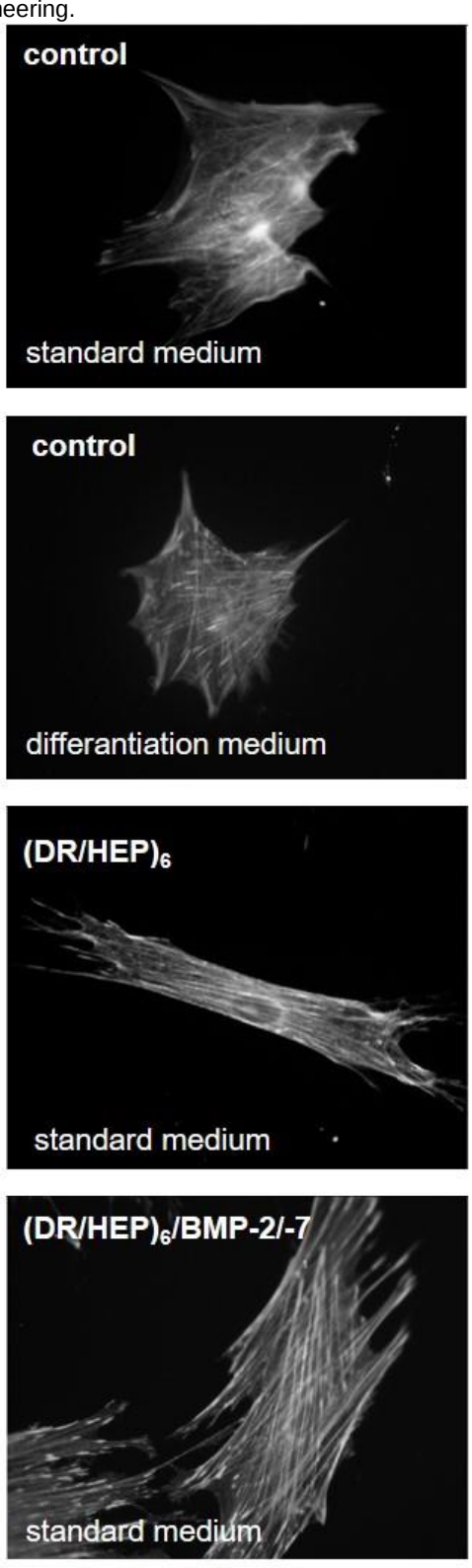

FIG. 1. hUC-MSCs cytoskeleton organization (fluorescence of F-actin, winkulin, nucleus) cultured in different environment.

Acknowledgments
This work was supported by the Polish National Science Centre, project no. 2016/21/D/ST5/01636.

References
[1] X. Li, W. Yi *et al.*, Am. J. Transl Res. 7 (2015) 1417-1428.
[2] H. H. Luu, W. X. Song *et al.,* J. Orthop. Res. 25 (2007) 665-677

NANOHYDROGELS BASED ON SELF-ASSEMBLY OF CATIONIC CURDLAN AND ANIONIC HYDROXYPROPYLCELLULOSE DERIVATIVES FOR PIROXICAM DELIVERY

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[Engineering of Biomaterials 158 (2020) 17]

Introduction
Piroxicam is a nonsteroidal, anti-inflammatory drug (NSAID) belonging to the oxicam group. It shows analgesic properties, as well as the anti-inflammatory and antipyretic activity. Unfortunately, piroxicam is only sparingly soluble in water. We believe that an appropriate nanocarrier can effectively increase its bioavailability. Here, we propose self-organizing, polysaccharide-based, nanoparticulate system designed for piroxicam delivery.

Materials and Methods
^{1}H NMR, XPS and IR spectroscopy were used to characterise polysaccharides modified in order to obtain self-organizing system. The obtained nanoparticles were characterized using dynamic light scattering (DLS) and zeta potential measurements. The structure of the nanoaggregates was studied by SEM and AFM.

Results and Discussion
A nanoparticulate system based on the ionic modifications of natural polymers was obtained. Two derivatives of natural polysaccharides were successfully synthesized and characterized: cationic curdlan (modified with glcydyltrimethylammonium groups) and anionic hydroxypropylcellulose containing styrenesulfonate groups. Due to the polycation-polyanion interactions they spontaneously self-assemble into nanoparticles in water. The size and surface charge of the nanoparticles can be controlled by the polycation/polyanion ratio. The resulting structures are spherical, with diameters in the range of 200 -300 nm, as confirmed by AFM, SEM, and DLS measurements. The size of the nanospheres decreases in elevated temperatures. The binding constant (Ka) of piroxicam to the anionic hydroxypropylcellulose (HPC-SSS) was determined by spectrophotometric measurements. The value of Ka was calculated according to Benesi–Hildebrand equation to be Ka. = (2.6 ± 0.14) × 10^3 M^{-1}. Piroxicam was effectively entrapped inside nanospheres.

Conclusions
In conclusion, we have obtained a novel, self-organizing nanoparticulate system, based on natural polymers – curdlan and hydroxypropylcellulose. We believe it constitutes a promising carrier for piroxicam, as it provides hydrophilicity and protects the drug from the unfavourable conditions. Biological tests are in progress.

Acknowledgments
We thank the National Science Centre (NCN) for the financial support in the form of the project UMO-2014/15/D/ST4/02770.

TITANIUM ALLOY COATED WITH PHLOROGLUCINOL-ENRICHED COLLAGEN FIBRILS REGULATES OSTEOGENIC DIFFERENTIATION AND INFLAMMATION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 18]

Introduction

Osseointegration of titanium alloy (Ti6Al4V) is dependent on the promotion of osteoblastic differentiation and reduction of inflammatory response. Collagen coatings can promote adhesion, proliferation and differentiation of osteoblast-like cells [1] and can act as matrices for biological molecules, as polyphenols [2]. Phloroglucinol (PG), the building block of phlorotannins, marine-derived polyphenols, can reduce inflammation and oxidative stress which may hinder osteogenic differentiation [3,4]. Previous studies showed it may have antibacterial properties [5]. This work study the influence of PG-enriched collagen fibrillar coatings on Ti6Al4V on the inflammatory response of fibroblast-like cells and osteogenic differentiation of osteoblast-like cells.

Materials and Methods

Collagen hydrogels were prepared, as described previously [6]. Hydrogels containing PG were also prepared with PG solution of 0.333 mg/mL and 1.0 mg/mL. Ti6Al4V discs, produced as described previously [7], were coated by placing hydrogels on their surfaces, allowing fibrils' absorption. Coatings of collagen and collagen with the lower and higher PG concentrations were named: Ti_Col, Ti_Col_low and Ti_Col_high, respectively, and analyzed by Scanning Electron Microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS) and contact angle (CA) measurements. *In vitro* studies were performed with human osteosarcoma SaOS-2 and mouse embryonic fibroblast 3T3 cell lines and 5×10^4 cells/well were cultured for 3 days on each sample. The gene expression of inflammatory and osteogenic differentiation markers was calculated by real-time polymerase chain reaction.

Results and Discussion

SEM images (FIG. 1) illustrated the presence of collagen fibrils after washing which was confirmed by XPS and CA measurements (data not shown). *In vitro* studies showed that collagen-coated Ti with PG, seem to promote osteogenic differentiation, by an increase of *COL1A1* and *BGLAP* genes with a high PG concentration (FIG. 2a) which are markers of bone matrix production and mineralization regulation, respectively. The expression of *RANKL*, a marker of osteoclast activation and bone resorption, decreased with the coating but when PG concentration increased it increased as well (FIG. 2b). Regarding inflammatory markers (FIG. 3), the coatings reduced their expression. SEM images showed that osteoblast- and fibroblast-like cells attached and spread well on uncoated and coated Ti6Al4V (data not shown).

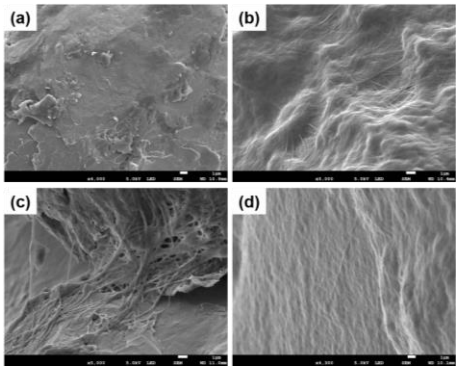

FIG. 1. SEM images (a) uncoated Ti, and (b) Ti_Col, (c) Ti_Col_low and (d) Ti_Col_high coatings.

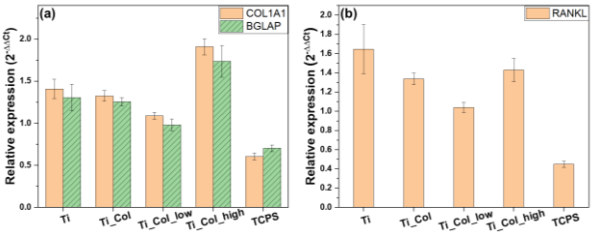

FIG. 2. Relative expression of (a) COL1A1 and BGLAP genes and (b) RANKL gene in osteoblast-like cells.

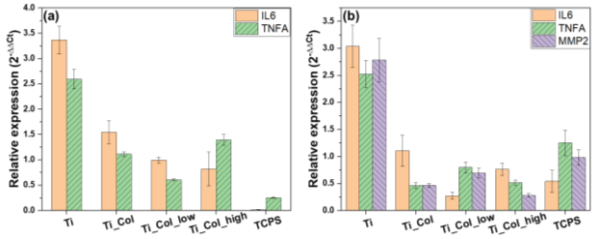

FIG. 3. Relative expression of inflammatory markers in (a) osteoblast-like cells and (b) fibroblast-like cells.

Conclusions

SEM, XPS and CA measurements confirmed the presence of the collagen coatings. *In vitro* tests revealed that PG-enriched coatings may reduce inflammation, promote osteogenic differentiation, and reduce osteoclast activation. Thus, PG-enriched collagen fibril coatings on bone implants are a promising approach.

Acknowledgments

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References

[1] U. Geißler, et al., J. Biomed. Mater. Res., 2000;51;4; 752–760.
[2] T.E.L. Douglas, et al., Biomacromolecules, 2007;8; 1085–1092.
[3] X.C. Bai, et al., Biochem. Biophys. Res. Commun., 2004;314;197–207.
[4] M. Yeo, et al., J. Mater. Chem., 2012;22;3568–3577.
[5] T.E.L. Douglas, et al., Biomed. Mater., 2016;11.
[6] M. Vandrovcova, et al., J. Biomed. Mater. Res. - Part A, 2015;103;525–533.

POLYMERIZATION SHRINKAGE OF NEW DENTAL COMPOSITES MODIFIED WITH LIQUID RUBBER

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[ENGINEERING OF BIOMATERIALS 158 (2020) 19]

Introduction

Light cured resin based composites (RBC) are used in dentistry from the 1970's. Their matrix is usually a mixture of dimethacrylate resins, while the reinforcement is ceramic particles. These components are supplemented by inhibitors, stabilizers, initiators, pigments etc., guaranteeing obtaining the desired functional and aesthetic features. Despite significant achievements in the field of modern dental composites, the problem of shrinkage still occurs, causing consequences in the form of marginal fissure, inflammation and secondary caries. The main factors that have a significant impact on shrinkage are the content and type of inorganic filler in the composite, the molecular weight and the degree of conversion of the monomer system [1]. In work [2], modification of the resin matrix was proposed through the use of bicyclic compounds (e.g. spiro orthoesters) in ring-opening polymerization. Liquid rubber has the effect of reducing polymerization shrinkage of epoxy resin [3]. However, despite attempts to toughening dimethacrylate resins contained liquid rubber [4,5], there are no such reports on changes in polymerization shrinkage of RBC.

Materials and Methods

Two commercial composites: Flow-Art and Boston (Arkona) was used for modification and testing. The matrix was a mixture of dimethacrylate resins: Bis-GMA, TEGDMA, UDMA and EBADMA. Composition of the mixture was completed by the addition of photoinitiator, stabilizer and inhibitor. Both types of composites contained the same ceramic filler which was a mixture of Ba-Al-B-Si glass, pyrogenic silica and titanium dioxide. The flow type composite contained 60% ceramics by weight of polymer matrix, while packable composites contained 78% wt. of reinforcement. The exact amounts of ingredients and their composition were patented by the manufacturer (Arkona). The modification of RBC's was made by addition of 5% by weight (of resin) of a liquid poly(acrylonitrile-co-butadiene) copolymer Hypro® 2000X168LC VTB (CVC Thermoset Specialties, USA). The following material designations were adopted: F – Flow Art, B – Boston and FM and BM – modified F and B composites, respectively.

Polymerization shrinkage measurements were carried out using the own method using computed microtomography (Skyscan 1174, Bruker microCT) [6]. A sample of the material was applied to a pin with diameter of 5 mm made of PTFE. During the scan, the pin with the material rotated in the half-angle range with a 9° step, 20 images were taken with a resolution of 6.6 μm. Immediately after scan polymerization was performed using the LED lamp of intensity 1350 mW/cm^2. The second scan with the same settings was done after 2 minutes from polymerization. The volumetric shrinkage was calculated as the ratio of the difference between uncured and cured material volume to uncured composite volume. Each composite was measured 10 times and results were statistically analyzed using Statistica software (TIBCO Software Inc.).

Results and Discussion

Result of shrinkage measurements were presented in FIG. 1. Modification of the matrix of composites with liquid rubber significantly reduced the level of volumetric shrinkage for both flow and condensable materials. Material F showed a shrinkage of 3.96% ± 0.41, while modification with liquid rubber allowed to reduce this value by over 6%. Similarly, in the case of material B, for which the measured polymerization shrinkage was 2.87% ± 0.28, after modification its value decreased by over 10%. According to work [7] the reactive rubber can be partly embedded in the crosslinked resin phase, which has reduced shrinkage. A possible explanation is the increasing content of carboxyl groups, which can catalyze the formation of ether reducing the network density.

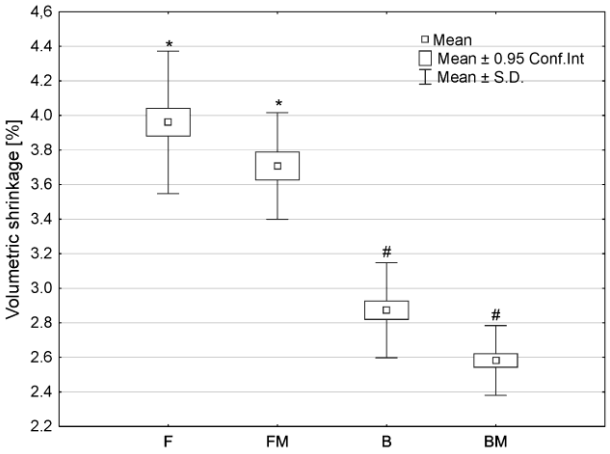

FIG. 1. Volumetric shrinkage of tested composites. Symbols (*) and (#) denotes significant differences between F / FM and B / BM composites, respectively.

Conclusions

Modification of dental composites with liquid rubber enables significant reduction of polymerization shrinkage. Addition of 5% by weight (of resin) resulted in decreasing of shrinkage by 6% for the Flow Art composite and 10% for the Boston condensable composite.

Acknowledgments

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References

[1] Kleverlaan C. J., Feilzer A. J., Polymerization shrinkage and contraction stress of dental resin composites. Dent. Mater. 2005, 21, 1150–1157.
[2] Bailey W. J., Cationic Polymerization with Expansion in Volume. J. Macromol. Sci. Part A - Chem. 1975, 9, 849–865.
[3] Kaynak C., Ozturk A., Tincer T., Flexibility improvement of epoxy resin by liquid rubber modification. Polym. Int. 2002, 51, 749–756.
[4] Kerby R. E., Tiba A., Knobloch L. A., Schricker S. R., Tiba O., Fracture toughness of modified dental resin systems. J. Oral Rehabil. 2003, 30, 780–784.
[5] Matsukawa S., Hayakawa T., Nemoto K., Development of high-toughness resin for dental applications. Dent. Mater. 1994, 10, 343–346.
[6] Pałka K., Zaszczyńska A., Kleczewska J., Polymerization shrinkage of dental composites. Eng. Biomater. / Inżynieria Biomateriałów 2017, 20, 62.
[7] Pionteck J., Müller Y., Häußler L., Reactive epoxy-CTBN rubber blends: Reflection of changed curing mechanism in cure shrinkage and phase separation behaviour. Macromol. Symp. 2011, 306–307, 126–140.

PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL TESTS OF SILK FIBROIN/COLLAGEN/CHITOSAN MATERIALS, CROSS-LINKED BY GLYOXAL SOLUTION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 20]

Introduction

The large molecular compounds, derived from natural sources, are characterized by properties desired in tissue engineering: biocompatibility, biodegradability, lack of immune responses after introduction into the human body. Currently, polymer implants are most commonly manufactured with chitosan and collagen [1,2]. However, this kind of materials exhibit poor stability in water conditions, and poor mechanical properties. Therefore, it is necessary to search for new materials that can potentially be used in tissue engineering. For the production of biomaterials with better properties, mixtures of two or more biopolymers should be used [3]. Significantly better mechanical properties have been demonstrated for silk fibroin [4]. Silk fibroin is a protein component of raw silk, where it performs structural functions [4]. It was decided to create ternary mixtures based on the three polymers (silk fibroin, collagen and chitosan). In addition, such mixtures can be subjected to cross-linking process, to improve parameters such as stability in water conditions and regularity of pores. This type of modification is expected to improve the mechanical properties of materials, stability in water conditions and degradation resistance [5].
Glyoxal is a very simple organic compound with two aldehyde groups. It is the smallest dialdehyde. Glyoxal is a really common cross-linking agent of polysaccharides and proteins [6,7].

Materials and Methods

Chitosan (CTS) was supplied by Sigma-Aldrich (Poznań, Poland). Silk fibroin and collagen were obtained in house. Collagen was obtained from young rat tail tendons. Silk fibroin was prepared from Bombyx mori cocoons (Jedwab Polski Sp. z o.o., Milanówek, Poland), as a 5% concentrated solution. Then the solution was filtered. Chitosan and collagen were prepared as 1% solution in 0.1 M acetic acid.
Three types of mixtures were prepared. The first type included chitosan and collagen (50/50 weight ratio) mixtures with 10, 20 and 30% silk fibroin addition. The second type corresponded to silk fibroin and collagen (50/50 weight ratio) mixtures with 10, 20 and 30% chitosan addition, and the third type concerned 50/50 weight ratio silk fibroin/collagen mixtures with 10, 20 and 30% of chitosan addition. The mixtures were poured into 24-well polystyrene culture plates, frozen, and lyophilized (ALPHA 1–2 LDplus, CHRIST, -55 °C, 5 Pa, 48 h).
The following properties of the materials were measured: density and porosity, moisture content and swelling degree. Mechanical properties of the 3D materials under compression were studied. Additionally, metabolic activity of MG-63 osteoblast-like cells on materials was examined.

Results and Discussion

It was found that the materials were characterized by a high swelling degree and good porosity, which can be suitable for tissue engineering applications. None of the materials showed to be cytotoxic to MG-63 cells.

Conclusions

Glyoxal solution was a good cross-linking agent for three dimensional materials based on the blends of silk fibroin, collagen and chitosan. It was found that cross-linked materials were characterized by high swelling rate and adequate porosity, which can be suitable for tissue engineering applications. Mechanical properties vary depending on the blends composition. The highest Young's modulus among the studied scaffolds was observed for SF/CTS/10Coll scaffold. None of the studied materials was cytotoxic to MG-63 cells. The cross-linking of ternary biopolymer blends with glyoxal may be a new way of materials' modification which offers a cheaper alternative to the existing methods of chemical cross-linking.

Acknowledgments

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References

[1] S.V. Madihally, H.W.T. Matthew, Biomaterials 20 (1999) 1133-1142.
[2] J.A.M. Ramshaw, J.A. Werkmeister et al., Biotechnol. Genet. Eng. 13 (1996) 335-382.
[3] T.W. Chung, Y.L. Chang, J. Mater. Sci-Mater. M 21 (2010) 1343-1351.
[4] A. Sionkowska, Prog. Polym. Sci. 36 (2011) 1254-1276.
[5] J. Skopinska-Wisniewska, J. Kuderko et al., Mat. Sci. Eng. C 60 (2016) 100–108.
[6] J. Berger, M. Reist et al., Eur. J. Pharm. Biopharm. 57 (2004) 19–34.
[7] S. Banerjee, J. Biosci. 45 (2020) 32.

MICROSTRUCTURE, SURFACE TOPOGRAPHY AND ADHESION OF ZEIN COATINGS ELECTROPHORETICALLY DEPOSITED ON TITANIUM SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 21]

Introduction

Titanium and its alloys are characterized by relatively good biocompatibility, high corrosion resistance, favourable fatigue strength and high strength to weight ratio [1]. They are commonly used as metallic biomaterials. However, this material is inert to our body and has poor osteoinductive properties [2]. Another significant problem is biofilm formation on implants [3]. |To solve these problems, polymer and composite polymer-based coatings are deposited on their surfaces. One promising polymer is zein. Zein is a natural protein polymer obtained from corn. Due to its biocompatibility, biodegradability and antibacterial ability, this material is growing in interest for biomedical applications [4]. A convenient method for the deposition of zein coatings on titanium substrates is electrophoretic deposition (EPD). The aim of this work was to investigate the influence of the chemical composition of the suspension and EPD parameters on the coating morphology and surface topography, as well as adhesion to the titanium substrates.

Materials and Methods

A commercially pure titanium (CP-Ti) Grade 1 was used as the substrate material for the EPD of zein coatings. The samples in the as-received condition were washed with distilled water and degreased with technically pure ethanol prior to deposition. In addition, some samples were chemically treated in the following way: washing in acetone, soaking in a 0.06 M $Na_3PO_4 \cdot 12H_2O$ solution at 80 °C, washing in hot water, soaking in an acid solution (5 ml HF 40 vol. % + 35 ml HNO_3 70 vol. % in 60 ml H_2O) for 5 min., washing in water and drying.

To prepare a suspension for the EPD process, zein powder in various concentrations (100, 150 or 200 g/l) was gently added to the solutions of anhydrous ethanol (75 or 80 or 90 vol. %), glycerol (20 wt. %) and distilled water (25, 20 or 10 vol. %, respectively).

An austenitic stainless steel plate (X2CrNiMo17-12-2) was used as a counter electrode in the EPD cell. The distance between the electrodes was 10 mm. The deposition time was 5 minutes. The applied voltage was in the range of 3-10 V.

The as-deposited coatings were subjected to initial macroscopic observation and then the morphology of selected samples was examined by scanning electron microscopy (SEM). Phase identification was performed by grazing incidence X-ray diffractometry (GIXRD). The coating microstructure was investigated by transmission electron microscopy (TEM) on lamellae prepared by FIB from a cross-section. The surface topography of coatings was examined using atomic force microscopy (AFM) and optical profilometry. The adhesion of coatings to the substrate was investigated using the cross-cut tape-test, in accordance with ASTM D3359-17.

Results and Discussion

The coatings deposited on titanium substrates from suspensions containing 90 vol. % of ethanol, 10 vol. % distilled water, 20wt.% of glycerol, 150 g/l or 200 g/l of zein were macroscopically uniform. They were very similar macroscopically to each other and the value of voltage during EPD did not noticeably affect the quality of the coatings. Meanwhile, the coatings deposited from suspensions of different compositions were morphologically inhomogeneous with the presence of microcracks.

It was found during the tape-tests that, among all the coatings, the coating deposited from the suspension containing 200 g/l of zein, 90 vol. % of ethanol, 10 vol. % distilled water and 20wt.% of glycerol exhibited the highest adhesion (class 4B) to the as-received substrates. Moreover, the adhesion of this coating to the chemically treated substrates was much lower (class 0B). Thus, this coating was selected for further investigation.

The coating deposited at a voltage of 5 V during 300 s was dense and relatively homogeneous. However, open pores with a diameter in the range of 1-25 µm sporadically occurred. The microstructure of the coating observed with the use of TEM was dense. The thickness of the coating was 4.4 µm. It could be observed that, between the coating and the titanium substrate, a passive oxide layer with the thickness of ~10 nm was present. The GIXRD pattern revealed the presence of an amorphous zein phase in the coating.

The coating was relatively smooth. Its surface topography was different from the surface topography of the substrate. Typical surface topography parameters of the coating had the values: R_a (average roughness) = 234 ± 64 nm, R_q (mean square roughness) = 285 ± 75 nm and R_{max} (total roughness) = 1272 ± 266 nm, while those of the substrate material were as follows: R_a = 419.8 nm, R_q = 572.9 nm and R_{max} = 22.8 nm.

Conclusions

EPD conditions for the deposition of pure zein coatings on titanium substrates were elaborated. The coatings were dense and relatively homogeneous with few open pores. The coatings deposited from the suspension containing 200 g/l of zein in a dispersion phase composed of 90 vol. % of ethanol, 10 vol. % distilled water and 20 wt. % of glycerol, at 5 V during 300 s had the highest adhesion to the titanium substrate in the as-received condition. It was found that the coating exhibited poorly developed surface topography.

Acknowledgments

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References

[1] M. Geetha, U. Kamachi Mudali, A.K. Gogia, R. Asokamani, B. Raj, Corrosion Sci. 46 (2004) 877-892
[2] M. Tamaddon, S. Samizadeh, L. Wang, G. Blunn, C. Liu, Int. J. Biomater. Tom (2017) 1-11
[3] K.D. Secinti, H. Özalp, A. Attar, M.F. Sargon, J. Clin. Neurosci. 18 (2011) 391-395
[4] S. Kaya, A.R. Boccaccini, J. Coat. Technol. Res. 14 (2017) 683-689

FABRICATION AND CHARACTERIZATION OF MULTICOMPONENT HA/MoS2/PEEK COATINGS ON THE Ti-13Nb-13Zr ALLOY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 22]

Introduction

Among all structural metallic materials, titanium alloys are the most prominent in contemporary implantology. They are characterized by a high strength to weight ratio, superior electrochemical corrosion resistance and a relatively low modulus of elasticity [1]. However, titanium alloys, including the Ti-13Nb-13Zr alloy, exhibit relatively poor tribological properties and are inert to the human body. Polyetheretherketone (PEEK) is a promising material for a bioactive coating matrix. PEEK is a semi-crystalline thermoplastic polymer with good tribological properties and relatively high strength. It is commonly used in biomedical engineering as a replacement for metallic, orthopaedic, long-term implants due to its high performance and biocompatibility [2]. Despite its advantages, its bioactivity is low but may be improved by the addition of bioactive particles. Synthetic hydroxyapatite (HA) is a commonly known and widely applied osteointegrated agent. It is a primary non-organic bone component. The effectiveness of the bone bonding capability and enhanced implant stabilization of HA have been widely proven clinically [3]. To elicit the bioactive properties of titanium alloy substrates, HA nanoparticles and MoS_2 nanosheets were co-deposited with PEEK microparticles in this work.

The goal of the present work was the fabrication of multicomponent HA/MoS_2/PEEK coatings on the Ti-13Nb-13Zr alloy through duplex treatment consisting of electrophoretic deposition (EPD) and post heat treatment. The microstructure and adhesion of the coatings to the titanium alloy were also characterized.

Materials and Methods

Composite coatings were deposited by EPD on Ti-13Nb-13Zr alloy disks with a diameter of 27 mm and thickness of 4 mm. Specimens were ground with 1200 grit sandpaper and subsequently washed in distilled water and ethanol.

The HA used for EPD was delivered in the form of nanopowder with elongated particles and with the average size of 43 nm and specific surface area of 46 m^2/g. To prepare a suspension for the EPD of coatings, 1.5 g of PEEK704, varying amounts of HA nanoparticles of 1 g, 0.5 g or 0.3 g and 0.1 g with 0.02 g of MoS_2 powders in 50 ml of an electrolyte composed of ethanol and 25 vol. % of colloidal chitosan solution were mixed. The suspension was prepared gradually. Firstly, by dispersing the suspension containing PEEK and MoS_2 in an ultrasonic bath for 20 minutes. Then, by adding HA powder, magnetic stirring at 300 rpm for 10 minutes and dispersing for 5 minutes. Electrodes were immersed at a constant distance of 10 mm apart in the EPD cell.

Coatings were deposited at a constant voltage in the range of 50-120 V with 10 V changes and a constant deposition time of 30 s. The specimens were exposed to heat treatment at a temperature of 380ºC for 40 min and cooled with rate of 2ºC /min.

Coatings were subjected to initial macroscopic observation. After that, the morphology of selected samples was characterized by scanning electron microscopy (SEM). The chemical composition of coatings was investigated by Energy Dispersive X-ray Spectroscopy (EDS) microanalysis. A cross-cut tape test in accordance with ASTM D3359-17 was performed to investigate the adhesion of coatings to the substrate.

Results and Discussion

It has been observed that macroscopically homogeneous coatings were obtained for cathodic deposition in the voltage range of 80-100 V. Coatings deposited at voltages below 80 V were too thin and often inhomogeneous. Application of higher voltages resulted in the deposition of coatings with uneven thickness and numerous pores. Therefore, the voltage of 90 V was finally adopted as optimal for obtaining macroscopically homogeneous coatings. The optimal time of deposition was determined at 30 seconds. Coatings deposited below that time were too thin, often not completely covering the alloy sample. Longer deposition times increased the probability of inhomogeneity.

Macroscopic evaluation of the coatings deposited from the suspension containing 1 g or 0.5 g or 0.3 g of HA revealed numerous inhomogeneities, such as cracks and pores with a diameter up to 100 µm. SEM investigation of the coating morphology after heat treatment revealed net-shaped microcracks appearing on their surfaces. Moreover, many unmelted PEEK particles covered by HA nanoparticles occurred on the surface of the coatings. It is supposed that the HA nanoparticles hindered the melting of the PEEK particles. EDS microanalysis confirmed the presence of calcium, phosphorus, molybdenum, sulphur, oxygen and carbon in the coatings. The Ca/P atomic ratio in the HA particles evaluated from the EDS spectra was close to 1.6.

It was found that the coatings deposited from the suspension containing 0.1 g of HA at the voltage of 90 V during 30 seconds were macroscopically homogeneous without any defects. After heat treatment, the PEEK changed its morphology from particles to a continuous and dense coating matrix. The tape-test conducted for the heat-treated coating revealed that this coating exhibited very high adhesion (class 5B) to the titanium alloy substrate. Detailed investigation of the coating using SEM showed that the edges of the cut were continuous. No detachment of the square of the coating from the incision grid was observed.

Summary

Macroscopically homogeneous HA/MoS_2/PEEK coatings were successfully obtained by EPD from a suspension containing 0.1 g of HA at a voltage of 90 V, during the deposition time of 30 seconds. Heat treatment densified the coatings and significantly enhanced their adhesion to the titanium alloy substrate. Further optimization and characterization of the coating microstructure, surface topography and properties are in progress.

Acknowledgements

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References

[1] T. Moskalewicz, A. Kruk *et al*., Journal of The Electrochemical Society 166(6) (2019) D151-D161
[2] G. Zhang, H. Liao, *et al*., Wear 260(6) (2006) 594-600
[3] T. Moskalewicz, A. Łukaszczyk *et al*., Materials Chemistry and Physics 99 (2017) 144-158

TITANIA COATINGS PRODUCED BY ELECTROCHEMICAL AND CHEMICAL OXIDATION OF TITANIUM ALLOYS SURFACE AND THEIR APPLICATION IN CONSTRUCTION OF IMPLANTS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 23]

Introduction

The titanium alloys are widely used in the construction of implants, which complement the bone defects in patients e.g. after complicated spine surgery or bone fractures. Although titanium based implants are typically expected to be longlasting, the lack of the integration with the bone often leads to implant failure and to the need to replace it. Therefore, one of the key challenges in bone healing and regeneration is the engineering of an implant, which provides osteointegration with enhanced bioactivity and improves implant-host interactions. One of the surface modification directions aiming at enhancement of bioactivity and osteointegration, is the formation of TiO_2 based coatings of defined structure, architecture, physicochemical, and mechanical properties, on the surface of titanium based implants. The low cost of electrochemical and chemical oxidation methods, and easy production of titanium dioxide nanotubular coatings, possessing the high surface-area-to-volume ratio, the strong oxidizing properties, the chemical stability, non-toxicity, the good mechanical properties, the excellent corrosion resistance and the high biointegration activity, caused a wide interest of these implants surface modification methods. The evaluation of relationship between structure, morphology and biological activity of titania nanotube coatings (TNT), produced by electrochemical oxidation of the implant surface, and titania fibrous layers (TNF), produced by titanium alloy surface chemical oxidation, were the aim of our investigations.

Materials and Methods

The amorphous TNT coatings were produced based on previously optimized anodic oxidation procedure [1-3], using Ti6Al4V alloy substrates and potentials U = 5-40 V. The samples of TNF coatings on the surface of Ti6Al4V substrates were produced using chemical oxidation method [2]. The surfaces of the substrates were chemically etched in a ca. 5.8 M HCl or 2M HF, then samples were heated in 30% H_2O_2 solution at 358 K, for different oxidation times. Samples surface morphologies were studied using the scanning electron microscope with field emission. Analysis of Raman spectra and XRD patterns of produced titania coatings allowed their structure determination. The photobleaching properties of TNT and TNF coatings were studied in accordance to ISO 10678; 2010 by the degradation of methylene blue [2]. The nanoindentation and nanoscratch-test analysis were performed to nanomechanical properties determination of studied TNT and TNF samples. The MTT assay was used to test specimen's influence on cells proliferation (measured after 24 and 72 h) [1,3,4]. We have studied the proliferation level of three cell lines growing on the surface of tested nanocoatings, i.e. adipose-derived human mesenchymal stem cells (ADSC), MG-63 osteoblasts and L929 fibroblasts were seeded onto the autoclaved TNT and TNF nanocoatings. Analysis of SEM allows observing the morphology changes of ADSCs co-cultured with MG-63 osteoblasts or L929 fibroblasts on the surface of tested nanolayers.

Results and Discussion

Among the applied oxidation methods, the direct chemical oxidation of Ti6Al4V substrates surface led to the produce of hydrophobic fibrous TiO_2 coatings (TNF), which mechanical properties and the biointegration activity was better in comparison to titania nanotube layers (TNT) produced by surface anodization (FIG. 1).

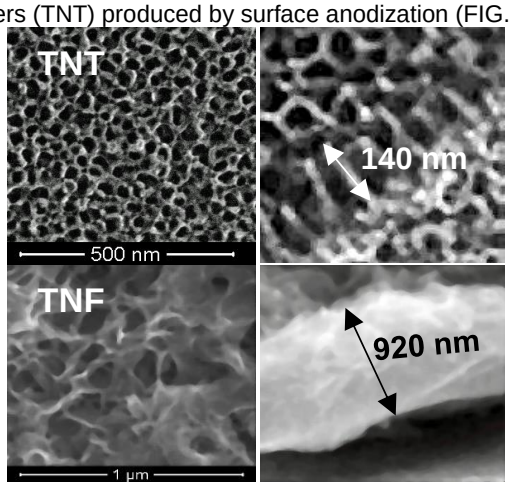

FIG. 1. SEM images of the surface morphology and cross-section of TNT and TNF coatings.

The results of nanoindentation studies proved that TNF coatings characterized good adhesion to the substrate surfaces, higher values of the Young's Modulus, and lower roughness. The viability level of all cell lines (mouse L929 fibroblasts, human osteoblasts-like MG-63 cells, and adipose-derived human mesenchymal stem cells (ADSCs)) increased after 72 h of culture on completely amorphous TiO_2 nanofibers surfaces versus TNT layers and the control sample (Ti6Al4V alloy). Simultaneously, it should be noted that also the photocatalytic activity of TNF coatings was higher in comparison to TNT ones. This would suggest that TNF coatings are also able to actively support the sterilization process of medical devices carried out in the presence of UVA radiation, increasing the speed and efficiency of the process of degradation of organic pollutants.

Conclusions

The research exhibited more favourable bioactivity and photocatalytic properties of titania nanofibrous coatings in comparison to titania nanotube ones. This suggest that the use of TNF layers obtained by chemical oxidation of the implant surface is more prove to be beneficial and can be applied as a novel alternative for bone tissue regeneration.

References

[1] Piszczek P, Radtke A, Ehlert M, Jędrzejewski T, Sznarkowska A, Sadowska B, Bartmański M, Erdoğan K Y, Ercan B, Jędrzejczyk W. J. Clin. Med. 9 (2020) 342.
[2] Ehlert, M.; Radtke, A.; Topolski, A.; Śmigiel, J.; Bartmański, M.; Piszczek, P. Materials 13 (2020), 2649.
[3] Ehlert, M.; Roszek, K.; Jędrzejewski, T.; Bartmański, M.; Radtke, Int. J. Mol. Sci. 20 (2019) 5642.
[4] Radtke A., Ehlert M., Jędrzejewski T., Bartmański M. J. Clin. Med. 8 (2019) 272.

COMPREHENSIVE EVALUATION OF THE BIOLOGICAL PROPERTIES OF SURFACE-MODIFIED TITANIUM ALLOY IMPLANTS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 24]

Introduction
Recent efforts in the field of implantology have highlighted the significance of modifying implant surface topography and biomaterial composition to improve their biocompatibility. Titanium and its alloys are commonly used as biomaterials for orthopedic, dental or neuro-surgical applications. Even though titanium based implants are typically expected to last ten years or more, their longevity is not assured and the lack of integration into the bone for long-term survival often occurs and leads to implant failure. Therefore, a planned modification of the surface of the alloys is strived to obtain a highly biocompatible coating with a strictly defined structure and architecture.

Materials and Methods
In order to fabricate titania nanotube coatings (TNT) on the surface of Ti6Al4V substrates, the electrochemical anodic oxidation method was used. Ist generation titania nanocoatings were produced using an aqueous electrolyte solution - 0.3% HF and different anodizing voltage values: 5V (TNT5), 15V (TNT15) and 40V (TNT40). Samples were structurally and morphologically analyzed. They were also characterized in terms of wettability and mechanical properties. Biocompatibility of biomaterials was assessed on the basis of the degree of integration of MG-63 osteoblasts-like, L929 fibroblasts and adipose derived mesenchymal stem cells (ADSC) cultures on their surface *in vitro*. In the separate experiments, we investigated the effect of the tested nanocoatings on the proliferation level of MG-63 osteoblasts-like or L929 fibroblasts co-cultured with adipose-derived mesenchymal stem cells. MTT (mitochondrial enzyme activity) assays were used to evaluate tested specimen's influence on the cell proliferation after 24 and 72 h.

Results and Discussion
The goal of the presented study was to optimize the production of titania-based biomaterials with high porosity and defined nanostructure, which supports the cell viability and growth. We assessed the bioactivity of amorphous titania coatings of different nanoarchitectures (nanoporous, nanotubular and nanosponge-like) (TNTs), produced on the surface of Ti6Al4V alloy by electrochemical oxidation. Cell adhesion is more difficult on smoother surfaces due to the smaller actual surface than in the case of rough substrates. In the presented studies, the modification caused an increase in surface roughness and studies using fibroblasts and osteoblast correlate with the results of AFM causing an increase in cell proliferation with an increase in the S_a parameter.

Regarding the examination by SEM, it was observed that ADSC cells had a typical spindle shape and grew evenly on the entire surface of the nanocoatings. Importantly, ADSCs formed filopodia, which effectively attached the cells to the scaffolds surface despite its hydrophobic nature. It can be concluded that the nanoporous surface is favorable for ADSCs. ADSCs cells cultured on the scaffolds alone or co-cultured with MG-63 osteoblasts also produced extracellular matrix thus functionalizing the nanocoatings.

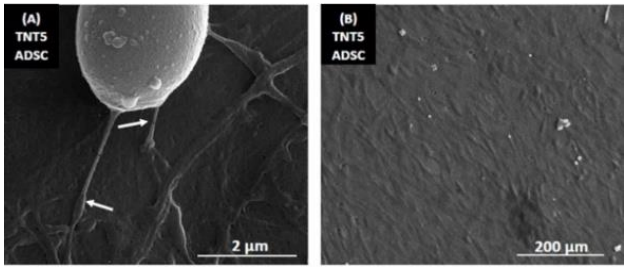

FIG. 1. Scanning electron microscopy (SEM) images showing adipose derived human mesenchymal stem cells (ADSC; A-B). Arrows in image A indicate filopodia attaching ADSC to the surface. The type of sample and scale of the images are shown in the figures as indicated.

Conclusions
The results of our experiments proved that the nano-porous surface is favorable for ADSC, which produced huge amounts of extracellular matrix when they were cultured on the scaffolds alone or co-cultured with MG-63 osteoblasts. The number of osteoblasts seeded and cultured with ADSCs on TNT5 surface after 72h culture almost doubled when compared with unmodified scaffold and rose by 30% when compared with MG-63 cells growing alone.

References
[1] Piszczek P., Radtke A., Ehlert M., Jędrzejewski T., Sznarkowska A., Sadowska B., Bartmański M., Erdoğan K.Y., Ercan B., Jędrzejczyk W. J. Clin. Med 9 (2020) 342.
[2] Ehlert M., Roszek K., Jędrzejewski T., Bartmański M., Radtke A. Int. J. Mol. Sci 20 (2019) 5642.
[3] Radtke A., Grodzicka M., Ehlert M., Jędrzejewski T., Wypij M., Golińska P. J. Clin. Med. 8 (2019) 334.
[4] Radtke A., Ehlert M., Jędrzejewski T., Bartmański M. J. Clin. Med. 8 (2019) 272
[5] Radtke A., Ehlert M., Jędrzejewski T., Sadowska B., Więckowska-Szakiel M., Holopainen J., Ritala M., Leskelä M., Bartmański M., Szkodo M., Piszczek P. Nanomaterials 9 (2019) 123.
[6] Radtke A., Grodzicka M., Ehlert M., Muzioł T., Szkodo M., Bartmański M., Piszczek P. Int. J. Mol. Sci 19 (2018) 3962.

THE BACTERICIDAL DEGRADATION EFFECTIVENESS OF TANNIC ACID-BASED THIN FILMS FOR WOUND CARE

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[ENGINEERING OF BIOMATERIALS 158 (2020) 25]

Introduction

Chitosan and tannic acid have been reported as active compounds with antimicrobial properties. The aim of the experimental study was to detect a potential synergy of antibacterial properties of chitosan and tannic acid against biofilm formation. The concentration of tannic acid released from material after immersion in SBF, SGF (simulated gastric fluid), SIF (simulated intestinal fluid) was determined by spectrophotometric method. Also, microbiological studies were carried out as an inhibition of bacteria growth test and bacteria adhesion observation. The obtained results allow for the selection of the optimal composition of the chitosan and tannic acid films, which will ensure adequate properties and bactericidal effectiveness.

Materials and Methods

Chitosan and tannic acid were purchased from Sigma-Aldrich company (Germany). Chitosan (CTS; DD=78%, Mv=1.8 × 10^6, shrimp derived) and tannic acid (TA; Mv=1701.2 g/mol) were dissolved in 0.1M acetic acid, separately, at a concentration of 2%. Complexes of chitosan and tannic acid were prepared in the weight ratios of 80/20 and 50/50, based on the previous research [1]. Thin films were prepared by solvent evaporation.

Tannic acid release

Tannic acid release was carried out in three different types of conditions - simulated body fluid (SBF; pH=7.4), simulated gastric fluid (SGF; pH=1.2) and simulated intestinal fluid (SIF, pH=6.8) which contained corresponding digestive enzymes. Selected solutions were prepared as traditional media and reference the appropriate conditions for film testing [2]. The total content of polyphenols was determined by the Folin-Ciocalteu method.

Inhibition of bacterial growth

Inhibition of bacterial growth was evaluated by measuring the turbidity of cultured bacterial broth with the tested materials according to McFarland standards [3]. The optical density was measured using The DensiCHEK Plus (BioMerieux, USA) and the readings were made after: 0.5, 2, 4 and 6h. The maximum measuring range of this device is 4 McFarland index /MSi/.

Adhesion of bacteria to the surface

Evaluation of bacterial adhesion to the film surfaces was performed by immersing the specimens in a bacterial solution, drying, covering them by gold, and then assessing with the use of scanning electron microscope (LEO Electron Microscopy Ltd, England). The Staphylococcus aureus strain (ATCC 29213) with the initial concentration of 1x108 CFU/ml was added to 30 ml of the Tryptic Soy Bulion (Merck, Poland) and incubated with specimens at 37°C for 14 days. Before the tests, the films (n=3) were soaked in 70% EtOH for 1 h and then washed in a sterile phosphate buffer solution.

Results and Discussion

The concentration of tannic acid released was detected after films' immersion in three different media – simulated body fluid (SBF; pH=7.4), simulated gastric fluid (SGF; pH=1.2) and simulated intestinal fluid (SIF, pH=6.8). The released concentration was calculated per 1mg of film. Tannic acid was released firstly from the material surface and then after 4h, as a result of material swelling, more tannic acid was released. Higher concentration of tannic acid was noticed for materials based on chitosan and tannic acid in 50/50 ratio than for 80/20, which is similar to the film composition. Both types of films showed constant tannic acid release for 24-72h immersion time in SBF and SIF. In SGF, after 72h, maximum concentration of TA was noticed as a result of total material dissolution. In SBF and SIF conditions the films remained in a solid form. Thereby, the obtained materials are proposed to be applied in contact with body fluids or in intestinal parts (pH around 7). In stomach-like conditions, the proposed materials would totally dissolve, which may be beneficial for drug delivery purposes. The results showed that the released tannic acid concentration depends on the medium's pH as well as on time of contact.

The prepared films were immersed in the bacterial solution of *Staphylococcus aureus* and their effect on the multiplication of bacteria was evaluated (table below).

Time:	*Staphylococcus aureus* stain (ATCC 29213)			
	K	The films composition		
		100CTS	80CTS/20TA	50CTS/50TA
0h	1.5			
2h	2.93	2.14#*	2.51#*	2.85#*
4h	>4	3.47#*	3.86#*	>4
6h	>4	>4	>4	>4

*Statistical analysis was performed between groups and control after 24h and the group, where the statistically significant difference occurred was marked.
max. SD ± 0.05

The prepared films were immersed in a bacterial solution and the adhesion of bacteria to their surface was evaluated (FIG. 1).

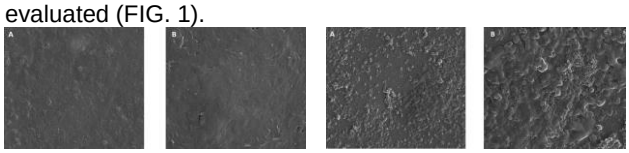

FIG. 1. The SEM images of films based on chitosan (left) and chitosan and tannic acid mixed in 50/50 ratio (right) with bacteria in magnification a) 10 000x b) 25 000x

Conclusions

Higher chitosan content resulted in the increase of bacteria growth inhibition. The burst effect of tannic acid release was noticed, which suggests that the obtained films may be beneficial for the pharmaceutical application. Based on the results, we believe that these chitosan/tannic acid films could be potentially used as wound dressing materials.

References

[1] B. Kaczmarek, et al. Mater. Lett. 245 (2019) 22-24.
[2] S. Sarıyer, et al. J. Drug Del. Sci. Technol. 56 (2020) 101551.
[3] M07: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th Edition.

PILOT STUDIES OF BIODEGRADABLE IRON-BASED 3D SYSTEMS – FOR THE NEEDS OF MODERN CARDIOLOGY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 26]

Introduction

The current stent technology is based on the use of permanent stent made from 316L stainless steel, nitinol and cobalt-chromium alloy, which are corrosion-resistant systems [1]. The implantation of bare metal stents has shown tremendous superior effects in various kinds of clinical situations, especially in the field of percutaneous coronary intervention, however they have specific properties, which limit their more widespread use. Since the major effect of stent implantation is provided by its scaffolding effect, it is required to last 6-12 months during which arterial remodeling and healing is achieved. After this period, the presence of stents within the body cannot provide any beneficial effects [2]. Thus the development of biodegradable stents, which can fulfill the mission and step away, is the logical approach. A near two decades long investigations into bioabsorbable stent materials have included both polymeric and metallic materials. Poly-L-lactic acid (PLLA) has been shown to possess acceptable biocompatibility, but a polymeric stent requires a greater strut thickness than most metal stents because of the polymer's lower ultimate tensile strength [3]. In the case of metals, their degradability is closely related to their susceptibility to corrosion. And although corrosion is generally considered as a failure in metallurgy, the corrodibility of certain metals can be an advantage for their application as degradable implants. The candidate metallic biodegradable materials for such application should have mechanical properties ideally close to those of 316L stainless steel, in order to provide mechanical support to diseased arteries. Non-toxicity of the metal itself and its degradation products is another requirement as blood and cells absorb the material. Based on the mentioned requirements, magnesium-based and iron-based alloys have been investigated as candidates for biodegradable stents [4]. Unfortunately, magnesium alloys show a relatively high rate of degradation (they can dissolve within 60-90 days from implantation, which is premature for vascular stenting applications) and associated evolution of hydrogen gas, which has raised concerns cytotoxicity and systematic toxicity. Instead, the results of first iron stent implantation showed no significant evidence of either the inflammatory response or neointimal proliferation, and organ examination did not reveal any systematic toxicity. Iron is also interesting because of its mechanical properties (high radial strength, high elastic modulus, and high ductility), which let to maintain during the implantation without any failure. However, its faster degradation rate is desired. Various techniques have been used recently on pure iron, to enhance its physical and biological properties maintaining their mechanical properties.

Materials and Methods

The iron systems have been obtained by the replica method where the polyurethane (PU) foam template was impregnated with suspension of pure iron made of metallic powder, ethyl alcohol and polyvinyl alcohol (PVA) solution, which was used to increase a suspension viscosity. The PU foam has been cut in the form of cube and it was immersed into abovementioned Fe-suspension, to allow suspension to completely impregnate the PU foam surface. The excess suspension has been removed to prevent the blocking of the pores and to obtain a uniform coating. In order to adjust the sintering process of iron foam, the course of the thermal decomposition PU foam has been investigated using differential thermal analysis (DTA) and thermogravimetric analysis (TGA). Preliminary tests showed that for pure iron system slow heating rate (1-2°C/min) to 600°C should be applied to prevent the structure collapsing during the burnout of the PU foam and other organic matter. Subsequently, a heating rate of 5°C/min should be applied to the final sintering temperature of 950°C. After the furnace cooling, the consolidated iron foam, which was the replica of the PU foam template, has been obtained. The samples thus obtained were subjected directly to all necessary structural and morphological tests, such as IR spectroscopy, Raman spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). Analysis of physico-chemical properties, such as wettability and free surface energy of obtained iron-based materials has been carried out. Moreover, the static immersion and potentiodynamic polarization tests have been used as *in vitro* experiments to assess the biodegradation behavior of samples in a modified Hanks' solution, which ionic composition and concentration are close to those of human blood plasma. The sample for the static immersion has been cleaned with ethanol, dried and weighed. Then it was suspended in Hanks' solution at a temperature of 37°C for 14 days, after which, it was removed from Hanks' solution, dried and weighed. The average corrosion rate (ACR) has been calculated based on the mass loss using equation from *ASTM G31 Standard*:

$$ACR = 8.76 \times 10^4 \frac{W}{A.t.p}$$

where ACR is the average corrosion rate in millimetre per year (mm/year), W is the mass loss (g), A is the exposed surface area (cm^2), t is the time of exposure in hours (h) and q is the density of Fe - 7.87 g/cm^3.

Results and Discussion

Optimizing the synthesis parameters, it was possible to obtain iron 3D materials, which show the porosity on micrometric level, as it is visible on SEM images (FIG. 1).

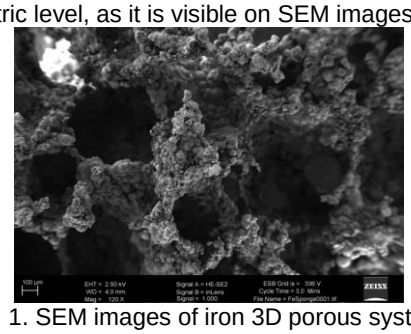

FIG. 1. SEM images of iron 3D porous systems.

The results of biodegradation behavior of sample will be presented during the conference.

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References

[1] P.K.Bowen,et al. *Adv. Healt. Mat.* 5(2016),1121-1140
[2] R. Waksman *J Invas. Card.*, 18(2006), 70-74
[3] C.M.Bunger et al.*J. Endovasc Ther*, 14(2007),725-733
[4] Moravej M., Mantovani D., *Int. J. Mol. Sci*, 12(2011), 4250-4270

THE ANTIMICROBIAL AND PHOTOCATALYTIC ACTIVITY EVALUATION OF THE POLYMER/TITANIUM(IV) OXO-COMPLEX COMPOSITES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 27]

Introduction

Antibiotic resistance is a problem that is increasingly beginning to affect us personally. This is the result of human action, which directly contributed to a drastic increase in the problem of antibiotic resistance. Unfortunately, it is not easy to overcome it, and one of the directions of research is to search for new materials showing high antimicrobial activity. The solution we propose is to use titanium(IV) oxo-complexes (TOCs). The aim of the study was the synthesis of (TOCs) with the $\{Ti_3O\}$ and $\{Ti_4O_2\}$ core as well as the synthesis of composite materials, by introducing oxo-complexes into the polymer matrix. The most important stage was to examine the microbiological and photocatalytic activity of the obtained composites. The structures we obtained have a chance to be used in the production of antibacterial surfaces.

Materials and Methods

Titanium(IV) oxo-complexes were synthesized as a result of the 4-hydroxybenzoic acid, 4-aminobenzoic acid and 9-fluorecarboxylic acid reaction with titanium(IV) isopropoxide as well as 4-hydroxybenzoic acid with titanium(IV) isobutoxide. Composite materials were produced by the incorporation of 20 wt.% oxo-complexes into the polymer matrix. The products were subjected to thermal analysis (STA 449 FS NETZSCH), infrared spectroscopy (FT-IR Spectrometer SPECTRUM 2000, Perkin Elmer), and Raman spectroscopy analysis (RamanMicro 200, Raman Microscope, Perkin Elmer). Microbiological tests were conducted for the following bacteria: *Escherichia coli*, *Staphylococcus aureus* and yeast: *Candida albicans*. The photocatalytic activity of produced composite materials was studied on the base of the methylene blue (MB) degradation procedure, in accordance to ISO 10678; 2010.

Results and Discussion

The oxo-complexes (TOCs) were synthesized in the direct reaction of titanium(IV) alkoxides ($Ti(O^iPr)_4$ and $Ti(O^iBu)_4$) and selected organic acids in 4:1 alkoxide/acid molar ratio, using a standard Schlenk technique under an argon atmosphere and room temperature (RT). The following organic acids were used in our experiments: 9-fluorenecoarboxylic acid ($HOOCC_{13}H_9$)), 4-aminobenzoic acid ($HOOC\text{-}p\text{-}PhNH_2$), and 4-hydroxobenzoic acid (HOOC-p-PhOH)). Analysis of IR and Raman spectra of synthesized oxo-complexes confirmed that their structure consists of $\{Ti_3O\}$ and $\{Ti_4O_2\}$ cores, according to the results of our earlier structural studies [1-3]. The photocatalytic activity estimation were carried our using polymer/TOCs composite foils produced by the dispersion of TOCs in the polymer solution (poly(methyl methacrylate) (PMMA)) and slow evaporation of the solvent. Scanning electron microscopy (SEM) confirmed the presence of uniformly dispersed microcrystalline powders of studied oxo-complexes in the composite films of 25-50 μm thick. The photocatalytic activity of synthesized trinuclear Ti(IV) oxo-complexes have been estimated basing on the UV photoinduced degradation process of methylene blue (MB), in accordance to ISO 10678; 2010 standard. The results of experiments carried out confirmed that all produced materials revealed the photocatalytic activity, however the best activity was found for $PMMA/[Ti_4O_2(O^iBu)_{10}(O_2C\text{-}PhOH)_2]$ system.
Microbiological studies proved that the composites obtained have good antimicrobial properties, each of the complexes tested caused a decrease in the number of microorganisms (TABLE 1).

TABLE 1. Antibacterial and antifungal properties of the PMMA film enriched with oxo-clusters, tested using the *S. aureus*, *E. coli* strains as well *C.albicans* ATCC 10231, in accordance with the EN ISO 22196 standard. The degree of microbial reduction [%]

	PMMA	PMMA+4-amino {Ti3O}	PMMA+4-hydroxy {Ti4O2}	PMMA+9-fluoro {Ti3O}	PMMA+ TiO_2 + 4-hydroxy {Ti4O2}
E. coli ATCC 8739	28,57 %	99,71%	100,00%	83,07%	100,00%
E. coli ATCC 25922	- 10,00 %	99,95%	100,00%	96,50%	100,00%
S. aureus ATCC 6538	- 105,56%	100,00%	100,00%	99,73%	100,00%
S. aureus ATCC 25923	- 310,00%	100,00%	100,00%	96,60%	100,00%
C. albicans ATCC 10231	- 20,00 %	82,00%	100,00%	81,00%	100,00%

Conclusions

The research results showed that oxo-complex $[Ti_4O_2(O^iBu)_{10}(O_2C\text{-}PhOH)_2]$ exhibits the best photocatalytic properties (decolorization of MB solution in the presence of the polymer/$[Ti_4O_2(O^iBu)_{10}(O_2C\text{-}PhOH)_2]$ composite sample, which was irradiated by UVA light). Simultaneously, the microbiological assays revealed that this composite caused a reduction in the number of microorganisms by almost 100%, which indicate on the high antimicrobial activity of this material.

References

[1] M. Janek, T. Muzioł, P. Piszczek, *Polyhedron*, 141 (2018) 110–117.
[2] M. Janek, A. Radtke, T. Muzioł, M. Jerzykiewicz, P. Piszczek, *Materials*, 11 (2018) 1661.
[3] M. Janek, T. Muzioł, P. Piszczek, *Materials*, 12 (2019) 3195.

DUAL CROSS-LINKING AS A METHOD OF IMPROVING MECHANICAL PROPERTIES OF GELATIN-ALGINATE HYDROGELS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 28]

Introduction

Gelatin exists as a mixture of water soluble protein fragments, with the same amino acid sequences as collagen, from which it is derived [1]. Alginate is a naturally occurring anionic polymer typically obtained from brown seaweed. Both, gelatin and alginate, have been extensively investigated and used for many biomedical applications, due to their biocompatibility, low toxicity, relatively low cost, and mild gelation process [2]. That is why the hydrogels based on their mixtures have been particularly attractive in wound healing, drug delivery, and tissue engineering applications [3].

Materials and Methods

Both gelatin and alginate were dissolved in water and then mixed in different volume ratios, to finally obtain solution 6% gelatin and 2% or 1,5% sodium alginate in one mixture. Then two different crosslinkers were prepared. Squaric acid (SQ) and N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride and N-Hydroxysuccinimide (EDC-NHS) both at 1% and 2% (weight percent based on dry weight of the protein) were dissolved in water and added to final mixtures. Then the hydrogels were immersed in calcium chloride solution to cross-link alginate.

Elongation tests were carried out on fresh hydrogel samples cut into pieces about 1 cm thick. The mechanical properties were determined using Zwick & Roell Z 0.5 machine (Germany).

Results and Discussion

The presented method allowed to obtain stable gelatin/alginate hydrogels.

Mechanical tests show that the tensile strength of materials containing 2% sodium alginate cross-linked with EDC-NHS and SQ increased. However, the tensile strength of hydrogels based on gelatin and 1,5 % sodium alginate undergo irregular changes after cross-linking.

The hydrogels with higher amount of sodium alginate are more rigid. The elongation at breaking point is lower while the Young Modulus is much higher. Interestingly, the materials containing 2% sodium alginate became more elastic, but for hydrogels containing 1.5% polysaccharide, the cross-linking effect is the opposite in most cases.

Conclusions

Mechanical tests confirm the mixing of gelatin and 2% sodium alginate form strong, relatively rigid materials. The cross-linking process additionally increases mechanical properties of these gels. EDC-NHS as a crosslinker is more appropriate for creation of gelatin/alginate hydrogels with improved mechanical properties.

Acknowledgments

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References

[1] X. Lou; T.J. Chirila, Biomater. Appl. 14 (1999) 184–191
[2] K.Y. Lee; D. J. Mooney, Prog. Polym. Sci. 37 (2012) 106–126
[3] S. Jinchen; T. Huaping, Materials 6 (2013) 1285-1309

HYDROGEL-BASED MATERIALS FOR DELIVERY MEDICINAL PLANT EXTRACTS – PRELIMINARY STUDIES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 29]

Introduction

Calendula officinalis (pot marigold) is one of the major medicinal plants. The phytopharmacological studies of marigold flower extract confirm its anti-inflammatory and antiviral activity as well as antimicrobial properties. Therefore, the flower extract of this plant has been used for the treatment of burns, ulcers, skin inflammations, eczema, and wounds [1,2].

The pharmacological activity of *Calendula officinalis* flower extract could be enhanced by its encapsulation that controls the release of active compounds over time. Encapsulation technology is used for improving the long-term stability of active substances and enhancing and prolonging the effectiveness of active ingredients [3].

The main goal of this study was to design, prepare and characterize novel hydrogel materials with plant extract-loaded microcapsules incorporated into hydrogel structure. The skin reaction was examined after application of the obtained hydrogel.

This solution in the design of hydrogel materials may become the basis for a new dermatological formulation.

Materials and Methods

The microspheres were produced from gelatin [4]. Hydrogels were made from 2% carrageenan solution [5] with the addition of fish collagen [6]. To prepare microcapsule-loaded matrices, gelatin microspheres with pot marigold flower extract were mixed with carrageenan/ collagen solution and magnetically stirred for 30 min. Then, 10 ml of each mixture was poured into Petri dishes. Finally, hydrogels were immersed in 1 M solution of potassium chloride at the temperature of 2^{o}C for 24 hours.

The skin color after application of obtained hydrogel was examined using the colorimeter (Skin-Colorimeter CL 400, Courage + Khazaka, Köln, Germany) with the use of MPA-software. The influence of the hydrogel on the skin was determined before application, immediately after application and 15, 30, 60, 120 and 180 min after application of the samples. The skin parameters measurements were conducted on the volar forearm skin with participation of five probants with normal skin (women, aged 23–29) after written consent, in the laboratory in controlled temperature and humidity.

Results and Discussion

The measured skin color is expressed as an XYZ-value and was calculated into L*a*b related value. L* gives information about the black-white axis and skin brightness, while a* and b* are the coordinates in the color space—a* locates the values on the red-green axis, whereas b* shows the color position on the blue-yellow axis. Therefore, the a* values were considered in this research due to their correlation to skin redness, erythema, and microcirculation. Skin color results suggest that the application of carrageenan/collagen - based hydrogels with incorporated microcapsules did not damage, irritate skin, or cause erythema (skin redness).

Conclusions

The preliminary studies confirm that the obtained hydrogels are safe for the skin, they do not cause its irritation. Further studies will be devoted to assessing the parameters of *Calendula officinalis* flower extract release from microcapsules incorporated into carrageenan/ collagen hydrogel.

Acknowledgments

Financial support from the National Science Centre (NCN, Poland) Grant No UMO 2016/21/D/ST8/01705 is gratefully acknowledged.

References

[1] N. Jan, K.I. Andrabi *et al.,* Proc. Indian Natn. Sci. Acad. 83 (2017) 769-787.
[2] M. Butnariu, C.Z. Coradini, Chem. Cent. J. 6 (2012) 35.
[3] I.T. Carvalho, B.N. Estevinho *et al*., Int. J. Cosmet. Sci. 38 (2016) 109-119.
[4] J. Kozlowska, N. Stachowiak *et. al.*, Polymers 10 (2018) 456.
[5] J. Kozlowska, K. Pauter *et al.*, Polym. Test. 67 (2018) 7–11.
[6] J. Kozlowska, A. Sionkowska *et al.*, Int. J. Biol. Macromol. 81 (2015) 220-227.

PREPARATION AND CHARACTERIZATION OF BIO-HYBRID HYDROGEL MATERIALS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 30]

Introduction

In recent decades, research attention has been focused on the development of modern hydrogel dressings due to their open porous structure, moisture retention, and good mechanical strength, ensure an optimal environment for cell migration and proliferation [1-5]. Therefore, the main goal of the presented research was to obtain bio-hybrid hydrogel matrices modified with the carrier-drug system [2,3]. Active hydrogel dressings, currently available on the market, do not have additional medicinal substances, therefore, the authors were made attempts to introduce a carrier-drug system into the hydrogel matrix to improve the wound healing process and its recovery.

Materials and Methods

The first stage of research concerned the synthesis of thermosensitive polymeric carriers by radical polymerization reaction in optimally selected reaction conditions. Then, the active substance (hydrocortisone) was introduced into polymeric carriers - the amount of the drug was selected on the basis of ointments available on the market. The systems were subjected to DLS analysis to determine the average particle sizes. In a further stage, bio-hybrid sodium alginate/poly(vinyl alcohol) based hydrogel matrices modified with various carrier-drug systems were obtained. The conducted research included the assessment of physicochemical properties of obtained hydrogel matrices i.e. determination of gel fraction, degree of hydrogel swelling, degradation studies as a function of pH and conductivity changes of distilled water and simulated body fluid in time. Additionally, the chemical structure of obtained hydrogels was confirmed using FT-IR spectroscopic technique.

Results and Discussion

The size of the drug carriers is an extremely important parameter. In this study, the analysis of the average particle size of a thermosensitive polymer carrier was 118 nm. In turn, analysis of the encapsulated carriers showed that both the amount of drug introduced and the encapsulation time directly affect the average particle size. The thermosensitive carrier containing 25 mg of the drug is characterized by an average particle size of 391 nm, while after the introduction of 50 mg of the drug it increases to 617 nm. FIG. 1. shows the average particle size of a thermosensitive polymer carrier - drug system containing 50 mg of drug in varied mixing time.

The gel fraction value (%GF) represents the insoluble gel fraction as a result of inter-molecules crosslinking formation. The obtained bio-hybrid hydrogel matrices with the carrier-drug system are characterized by higher levels of crosslinking (approx. 65%) compared to materials containing no active substance (approx. 60%).

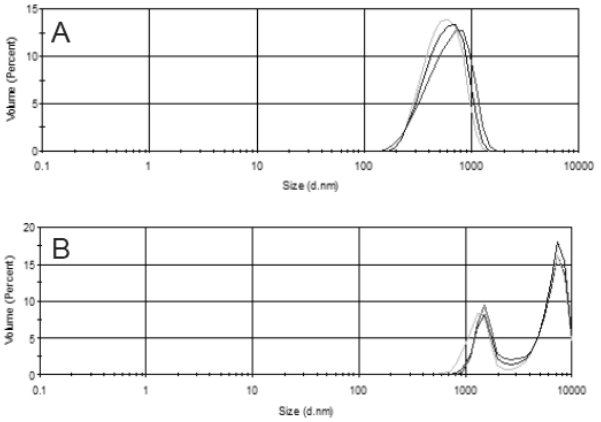

FIG. 1. The average particle size of the thermo-sensitive carrier-drug system, containing 50 mg of the drug after 10 min (A) and 24 h (B) mixing time.

The results of swelling tests indicate that the obtained bio-hybrid hydrogel matrices show a slightly higher absorption capacity in the water environment than the phosphate buffer. Based on the conducted research, it can be concluded that systems containing a higher concentration of the drug have greater sorption capacity in each of the media used. This is due to the fact that the packing density of the chains in the hydrogel matrix decreases as the additional components in the system increase. If a higher concentration of the drug is used, which is then released from the hydrogel matrix, additional gaps are created that can replace the absorbed fluid.

The analysis of FT-IR spectra confirms the chemical structure of the obtained bio-hybrid hydrogel matrices. In the case of modifications with a thermosensitive carrier, a much more intense band can be observed in the 3200-3500 cm^{-1} range, which most likely originates from the strong hydrogen interactions that occur between individual components. This is also confirmed by the determined %GF, which in the case of bio-hybrid matrices containing a thermosensitive carrier was the largest, which indicates a significant cross-linking of the matrix.

Conclusions

Based on the obtained results and observations, it can be concluded that the bio-hybrid hydrogel matrices are stable materials, and exhibit desirable features from the point of view of wound care applications. The conducted research may prove to be an important step in the fight against skin diseases and by developing new bio-hybrid hydrogel matrices modified with antibacterial agents, including antibiotics, nanoparticles, peptides or metal ions.

Acknowledgments

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References

[1] S. Utech, A.R. Boccaccini, J. Mater. Sci. 51 (2016) 271-310.
[2] E.A. Kamoun, E.R.S. Kenawy *et al.*, J. Adv. Res. 8 (2017) 217-233.
[3] T.R.R. Singh, G. Laverty *et al.*, CRC Press; 1 edition, 2018.
[4] M. Ding, L. Jing *et al.*, Mater. Today Adv. 8 (2020) 100088.
[5] A. R. Abbasi, M. Sohail *et al.*, Int. J. Biol. Macromol. 155 (2020) 751-765.

DESIGN AND MANUFACTURE OF CUSTOMIZED MEDICAL IMPLANTS - THIRD REPORT

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[ENGINEERING OF BIOMATERIALS 158 (2020) 31]

Introduction
Last year, we informed about the progress of our project POIR program 1 / 4.1.4 / 2017 financed by the National Center for Research and Development in Poland. The project concerns the design and manufacture of customized, osseointegrated percutaneous orthopaedic implants, intended for patients who have undergone an above the knee amputation. Such an implant allows load to be transferred directly from the femur to the prosthesis omitting soft tissues, that are usually involved in this process, when using a socket-suspension type prosthesis system. All planned work, both design and research, are currently advanced at around 80%. We plan to complete the project within the next year (2021).

Materials and Methods
Implants were designed using reverse engineering, biomodelling, and CAD software: Geomagic Design X (3D Systems, USA) industry standard reverse engineering software with advanced mesh editing tools; Solidworks (Dessault Systems Solidworks Corporation, USA); Geomagic Freeform (3D Systems,USA) a voxel based biomodelling software package that converts models into virtual clay that can be manipulated much like physical clay. All finite element analyses were performed using ANSYS R19.1 (ANSYS, Inc. Canonsburg PA, USA). The 3D implant project was analyzed from a manufacturing perspective using CAM software (Hypermill, OPEN MIND Technologies AG, Germany) and prototypes were manufactured form titanium alloy using a hybrid CNC milling system (Laser 1300, C.B. Ferrari, Italy). Sterilized implant prototypes were subjected to strength and fatigue tests in order to assess their suitability for long-term use. Microbiological environmental testing was carried out at the site where the implant prototypes were produced, so as to prepare and later implement procedures that would lead to a reduction of microbiological contamination. Implant prototypes were sterilized with using hot dry air method. Sterility of implant prototypes was checked by microbiological tests.
Thrombo-compatibility was assessed using scanning electron microscopy and flow cytometry tests, cytotoxicity was assessed by XTT test, and genotoxicity was assessed by micronucleus test. The carcinogenic potential of the materials intended for the manufacture of the implants was also evaluated. For this purpose, qRT-PCR technology was used to analyse the expression of selected genes involved in cancer processes. Primary and tumour lines of osteoblasts and chondrocytes were examined.

Results and Discussion
The production site where the implant prototypes were manufactured was subjected to microbiological control and the presence of a significant number of microorganisms was shown, both in the air and on work surfaces. Furthermore, particularly high concentrations of microorganisms were found in the cooling lubricants used in the CNC milling systems. Washing of the CNC machinery, coolant replacement and an improvement of sanitary procedures carried out by personnel resulted in a reduction of the number of microorganisms to an acceptable level. Using commercially available engineering programs, we successively designed 19 different versions of the implant prototype, that was fitted to the anatomical structures of an amputated femur. Subsequent versions took into account both feedback form the orthopaedic surgeons as well as the results of numerical analysis by the finite element method. The latest version of the prototype, following evaluation of microbial pollution and sterilization, was subjected to strength and fatigue testing. The fatigue testing results obtained to date have not been satisfactory, therefore several necessary changes will have to be introduced into the implant design. The hot dry air sterilization procedure proved to be efficient and very effective. We are currently awaiting the sterility results of the product after a shelf life of 3 and 6 months. Tests for thrombo-compatibility as well as cyto and genotoxicity evaluation of the materials, that will be used in the manufacture of the final medical devices, have been completed. None of the materials, nor any of the surface-modified samples, showed any adverse effects due to cyto and genotoxicity or due to thrombogenicity. The results of qRT-PCR analysis of the expression of selected genes involved in cancer processes are currently under analysis.
The clinical team has prepared a detailed surgical procedure that will be used to implant the devices. Whilst, the patient recruitment process to find suitable candidates for this procedure has commenced.

Conclusions
Despite numerous difficulties encountered during the implementation of the project the level of progress has been satisfactory and it will be possible to start work, in the nearest future, on personalized implants for specific patients. Next year, we should have the initial results of the first implemented procedures.

Acknowledgments
The project is financed by the National Center for Research and Development in accordance with the contract POIR.04.01.04-00-0058/17-00.

MAY METALLIC BIOMATERIALS USED FOR ORTHOPEDIC IMPLANTS PROMOTE CANCER PROCESSES? PRELIMINARY TRASRIPTOMICS RESEARCH ON HUMAN ARTICULAR CHONDROCYTES.

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[ENGINEERING OF BIOMATERIALS 158 (2020) 32]

Introduction

There are numerous reports about the formation of cancer changes adjacent to the implant or in places distant but temporally correlated with the implantation. This phenomenon is strongly marked in dental implantology, where one of the main types of cancer located in close proximity of dental implants is squamous cell carcinoma [1]. At the moment there is no indisputable data on the initiating of carcinogenesis by implants used in orthopedics, although this subject has been often discussed in works from the last three decades. For example, after total hip arthroplasty, the appearance of malignant neoplasms in the area of endoprostheses, including osteoma, osteosarcoma, lymphoma or squamous cell carcinoma has been reported [2,3]. However, no mechanism is fully confirmed, and the issue of accelerated tumour induction at the implantation site is still poorly understood and unclear. In the light of above information, our project assumes verification whether immortalized cell lines with neoplastic phenotype show an altered response to contact with implant material compared to primary cell lines of the same type.
In this report, the results from analysis of changes in expression of cancer-related genes in the human articular chondrocytes, performed by the use of qRT-PCR technique, have been presented.

Materials and Methods

The study was carried out for three types of materials used for implant production (medical steel AISI 316L, titanium alloys Ti6Al4V and Ti6Al7Nb). On the basis of the previously prepared literature review, 19 genes promoting cancer formation were selected and a custom PCR plate was designed. The primary chondrocytes of the HC-a line were purchased from ScienCell Research Laboratories (cat no. 4650). Six independent RNA isolation experiments from HC-a cells grown on the surfaces of the tested biomaterials were carried out using GeneMATRIX Universal RNA Purification Kit (EURx Ltd). The cells grown on the surface of a standard culture flask was used as a control.
Then, using Agilent's 2100 bioanalyzer, the quality and purity of isolated RNA was assessed. After this, with the use of the iScript cDNA Synthesis Kit (BIO-RAD), the reverse transcription was carried out.
The key stage of this study was to perform six qRT-PCR reactions using the CFX96 Touch thermal cycler (BIO-RAD) and 2xSsoAdvanced Universal SYBR Green Supermix reagent (BIO-RAD) on 96-well custom plates containing lyophilisates of 19 genes associated with development of tumorigenic processes.

Results and Discussion

The starting point for analysis of changes in gene expression in qRT-PCR is the Cq value, which is a measure of gene expression and can be defined as the number of cycles after which the signal exceeds the detection threshold. Differential analysis was performed, i.e. the expression of a examined gene in the tested sample (RNA from cells grown on the surface of biomaterials) was referred to the expression of this gene in the control sample (RNA from cells cultured without contact with the materials). The obtained results are presented as so-called ratio or fold change (Fc), i.e. the ratio of the Cq value of the sample to the Cq of the control. GAPDH and ACTB genes were selected as the reference genes.
The nonparametric one-way ANOVA test used for statistical analysis of results (statistical significance $p < 0.05$) indicated the occurrence of statistically significant changes of the average values of Fc for analyzed genes in the chondrocytes grown on the examined surfaces (AISI 316L, Ti6Al4V and Ti6Al7Nb) in comparison to control cells cultured without contact with any biomaterial. However, for a full interpretation of the results it is necessary to continue further studies including the analysis of changes in the expression of selected genes in chondrosarcoma cells (chondrocytes with neoplastic phenotype).

Conclusions

Analysis of changes in the expression of cancer-related genes plays a vital role in the assessment of risk of induction or intensification of carcinogenesis by the implants used in orthopedics. Compilation of qRT-PCR experiments carried out on primary and cancer cells in parallel will allow to identify possible future contraindications for patients with a genetic predisposition to cancer or with cancer history. What is more, this approach may be a crucial step in the selection of the right biomaterial for a specific patient.

Acknowledgments

This work has been entirely supported by the National Centre For Research and Development, project number POIR.04.01.04-00-0058/17-00.

References

[1] S. Jeelani, E. Rajkumar *et al*., J Pharm Bioallied Sci. 7(2) (2015) S378–S380.
[2] A.J. Smith, P. Dieppe *et al*., BMJ (2012) 344:e2383 doi: 10.1136/bmj.e2383.
[3] V. Levasic, I. Milosev *et al*., Acta Orthop. 89(2) (2018) 234–239.

DOES SURFACE STRUCTURING OF METALLIC MATERIALS AFFECT THROMBOCOMPATIBILITY?

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[ENGINEERING OF BIOMATERIALS 158 (2020) 33]

Introduction

Blood contact with biomaterial surface results in blood platelet adhesion, activation, and aggregation on the surface [1]. Growth factors released during platelet activation have a positive effect on osseointegration. Laser structuring of the surface of metallic biomaterials allows control of osteoblast proliferation and affects the formation of microbial biofilm on the surface [2]. The question arises what the impact of laser surface structuring on the thrombocompatibility of this surface is. The aim of this research was to determine the thrombocompatibility level of laser-modified metallic materials surfaces. Thrombocompatibility was assessed both on the surface of the laser-structured materials and also in the whole citrated blood after the contact with the tested surfaces.

Materials and Methods

Four types of materials were subjected to the thrombocompatibility test: AISI 316L, Ti6Al4V, Ti6Al7Nb, CoCrMo. The samples surfaces were structured differently: Series A - surface after machining (Ra 1.1 ÷ 1.2 μm), Series B - grinded surface (Ra 0.5 ÷ 0.8 μm), Series C and D - two different types of laser modification. Human citrated blood from healthy volunteers who did not take any drugs that affect platelet function within two weeks prior to the donation has been used. The studies have been approved by the Local Bioethics Committee at the Medical University of Lodz (no. RNN/46/06/KB 21.02.2006).

To assess the adhesion, aggregation and activation of platelets on the surface of tested materials, the samples were incubated for one hour with whole blood at 37°C, ensuring a gentle blood flow. Then, it was fixed in 2.5% glutaraldehyde and dehydrated in increasing concentration of ethyl alcohol (50%, 60%, 70%, 80%, 90%, absolute alcohol). This way prepared samples were sputtered with a 5-nanometer gold layer and imaged using a scanning electron microscope. Pictures were taken at random locations on each surface tested. Platelets were counted using the ImageJ software. The study of aggregation and activation of platelets in the whole citrated blood was examined using a flow cytometry technique. After one-hour incubation of citrated blood with the test samples, blood platelets were labeled with three fluorescence labelled antibodies: CD61-PerCP recognizing the CD61 platelet marker present on the platelet surface (β subunit of GPIIb/IIIa integrin); CD62-PE recognizing the CD62 receptor (P-selectin); FITC-PAC-1 recognizing the active form of fibrinogen receptor (GPIIb/IIIa). Blood samples were then fixed with CellFix reagent and analyzed on BD Accuri C6 or FACS ARIA flow cytometer (Becton Dickinson).

Results and Discussion

A contact of blood platelets with the artificial material surface triggers platelet activation, which is evidenced by platelet shape change. The stages of platelet activation, according to the Goodman's classification [3], were analyzed: from single no-activated platelets, through dendritic and dendritic-spread platelets (medium activation) to spread and completely spread with diffuse hyaloplasm (high activation). Platelet aggregation on the surfaces of the tested materials was assessed by counting small aggregates (up to 10 platelets) and large aggregates (above 10 platelets).

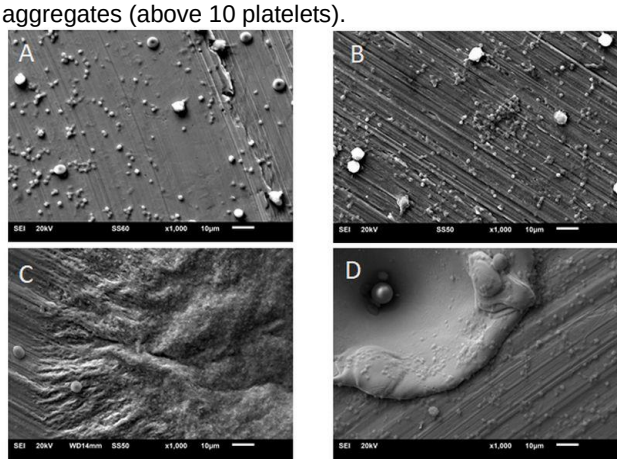

FIG. 1. SEM images of the surfaces with adhered blood platelets : A) AISI 316L, B) Ti6Al4V, C) Ti6Al7Nb, D) CoCrMo.

Flow cytometry studies allowed to determine the population of the aggregated platelets and the degree of platelet activation evidenced by the expression of two platelet activation markers.

Conclusions

Surface laser treatment within the D series for each of the tested materials promotes low activation of adhered blood platelets (the highest number of non-activated platelets) in comparison to the A, B and C series, especially for Ti6Al7Nb alloy.

The smallest number of aggregates in whole blood were observed after contact with grinded surfaces (Series B) of Ti6Al4V alloy, followed by the second titanium alloy Ti6Al7Nb.

All materials with C type modification caused the highest activation of platelets in comparison with other types of modifications. The lowest blood platelet activation in the whole blood was observed after the contact with B and D type modified materials surfaces.

Acknowledgments

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References

[1] M. Walkowiak-Przybyło, *et al*., Journal of Biomedical Materials Research - Part A, 2012 Volume 100 A, Iss. 3, 768-775

[2] P. Komorowski et al. J Biomed Mater Res. 2019; 1–11. https://doi.org/10.1002/jbm.b.34521

[3] Goodman S.L., Jouranl of Biomediacal Materials Research, 1999, 45(3), s. 240-250

DETERMINATION OF *ALOE VERA* RELEASE FROM ALGINATE BASED HYDROGELS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 34]

Introduction

New trends among hydrogel materials indicate their modification with active substances of both natural and synthetic origin, which allows for gradual and controlled release of the drug. The most important are hydrogels prepared based on biopolymers, such as: chitosan, alginate, gelatin or pectin. Therefore, the combination of alginate hydrogels with *Aloe vera* constitute a very interesting materials for application as wound dressings. *Aloe vera* extract/juice contains many active substances, due to it exhibits antibacterial, anti-viral, anti-inflammatory and anti-fungal activities. However, from the medical application point of view, the release profile of active substances, are very important and necessary. Properly designed hydrogels can provide controlled release of active substances, which increases the effectiveness of treatment, and the undesirable side effects of the used drugs reduces, significantly [1-4].

Materials and Methods

In this studies alginate based hydrogel matrix modified with *Aloe vera* (2% solution, in composition: 15%, v/v) were applied. This sample was obtained by radical polymerization and the initiator of the reaction was ammonium persulfate (APS), while poly(ethylene glycol) diacrylate (PEGDA) was used as the crosslinking agent. The gel fraction of this hydrogel matrix is around 60%. The chemical structure was confirmed using FT-IR spectroscopy. Moreover, the swelling abilities and other physicochemical properties of analyzed sample, were characterized. The release of active substance was conducted using USP4 method (DZF II Flow-Through System, Erweka GmbH, Langen, Germany) [5,6]. The equipment incorporated seven in-line flow-through diffusion cells (FIG. 1). The membrane was placed over a support with an orifice of 1,5 cm in diameter (diffusional area, 1.766 cm^2). The vertical cell was made in glass and was designed to have a volume into the donor compartment of 6.22 ml. All the cells were placed in a cell warmer connected with the Erweka heater DH 2000i and the Erweka piston pump HKP 720. The piston pump transports the receptor fluid via seven channels to the flow-through cells and automatically adopts the setting of the flow rate. All volumes were measured by gravimetric methods by filling the chambers with Milli-Q water and assuming a density of 1 g/ml. All the determinations were made in triplicate for each cell. The release study of *Aloe vera* was carried out using a regenerated cellulose membrane Spectra/Por®Dialysis Membrane MWCO 6-8,000 Carl Roth® Company. The assays were performed in aqueous medium as acceptor phases mimicking physiological conditions corresponding to buffer solutions at pH 7.4. A flow rate of receptor fluid of about 1 ml / 1 min. was selected. The experiment was carried out for 9 h, at 37ºC. Samples were evaluated at different time points, and data analysis was made by comparing the releasing efficiency (PE) values. The released concentration of *Aloe vera* in the receptor solution was analyzed by means of UV-Vis spectroscopy (Perkin Elmer Company), at the wavelength of 350 nm.

Results and Discussion

The obtained results exhibit that after first 2 hours of experiment burst release of *Aloe Vera* was reached, followed by the tendency to pulsatile active substance delivery to the acceptor fluid, was observed (FIG. 2). This is possible due to the chemical structure and crosslinking degree of the alginate based hydrogel.

FIG. 1. The equipment used for USP4 method (DZF II Flow-Through System, Erweka GmbH, Langen, Germany).

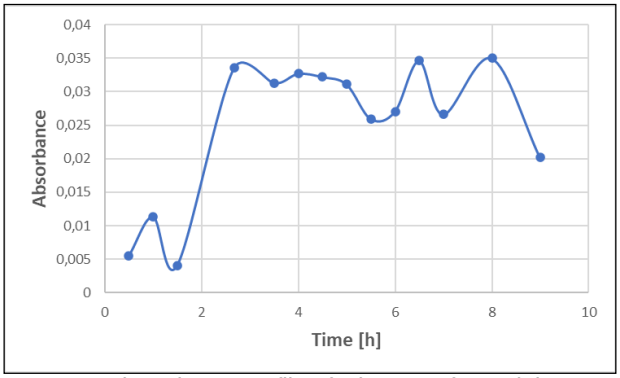

FIG. 2. The release profile of *Aloe vera* from alginate based hydrogel, at 37ºC.

Conclusions

The USP4 method constitutes the interesting alternative solution in the case of the determination of active substance release from different forms of drug, especially: hydrogel matrix, tablets, capsules, granules, ointments, suspensions, implants, stents, microspheres and suppositories. This method confirmed pulsatile mechanism of drug release form the hydrogel matrix which is useful for treatment of patients, due to the high efficiency and lack of undesirable adverse effects to the whole body.

Acknowledgments

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References

[1] R. F. Pereira, A. Mendes, and P. J. Bártolo, Chem. Eng. Trans., 32, 1009–1014, 2013.
[2] R. Pereira, A. Mendes, and P. Bártolo, Procedia CIRP, 5, 210–215, 2013.
[3] J. Leppiniemi et al., ACS Appl Mater Interfaces., 9 (26), 21959–21970, 2017.
[4] S. Rahman, P. Carter, and N. Bhattarai, J. Funct. Biomater., 8 (6),1–17, 2017.
[5] Farmakopea Polska X. Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, PTF Warszawa 2014
[6] R. Uddin, N. Saffoon, K. Bishwajit-Sutradhar, International Journal of Current Biomedical and Pharmaceutical Research, 2011, 1(4), 201-207.

STUDIES ON THE ENCAPSULATION OF MODEL ACTIVE SUBSTANCE USING THERMOSENSITIVE POLYMERIC NANOCARRIERS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 35]

Introduction
Currently, polymers are very important in human life, from everyday use to technologically advanced plastics. Stimuli-sensitive polymers are applied as intelligent systems for different purposes, namely thermosensitive polymers are notably widely used in biomedicine and pharmacy as drug delivery systems. The controlled release allows to deliver a drug to a specific location of the disease, reduce doses and side effects, shorten the time intervals when taking a drug (e.g. once a day), better protect the active substance and, as a result, increase the pharmaceutical and biological availability. Moreover, thermosensitive polymers are particularly interesting, because temperature changes can be applied there in a non-invasive way. An aqueous thermosensitive polymer solution exhibits reversible sol-gel transitions near the body temperature, which regulate the release rate of the introduced drug along with maintaining its physical-chemical stability and biological activity [1-5].

Materials and Methods
The carriers based on N-isopropylacrylamide were prepared by one-stage emulsion polymerization. The initial synthesis was the reaction of N-isopropylacrylamide with N, N'-methylenebisacrylamide in distilled water. After that, the chemical structure of the obtained polymeric particles was confirmed using FT-IR spectroscopy and the conversion was analyzed using UV-Vis spectroscopy. The average particle size of the thermosensitive carriers was determined based on the analysis of DLS results. Finally, the encapsulation of the model active substance – salicylic acid, was carried out. The salicylic acid belongs to nonsteroidal anti-inflammatory drugs with antiseptic and analgesic properties. In the next stage, the encapsulation efficiency was assessed and the average particle size of the carrier – drug system was analyzed. Additionally, SEM images of thermosensitive polymeric carriers before and after encapsulation were presented, and the chemical structure was compared on the basis of FT-IR spectra.

Results and Discussion
On the basis of the obtained DLS histograms we can conclude that the particle size of the carrier-drug system increased, but only slightly (in the range of 300-400 nm), compared to the polymer carrier - 118 nm, which was difficult to gain. The results regarding the encapsulation efficiency exhibited that the higher efficiency (about 45%) was obtained for samples prepared by sonication and using a lower concentration of salicylic acid.

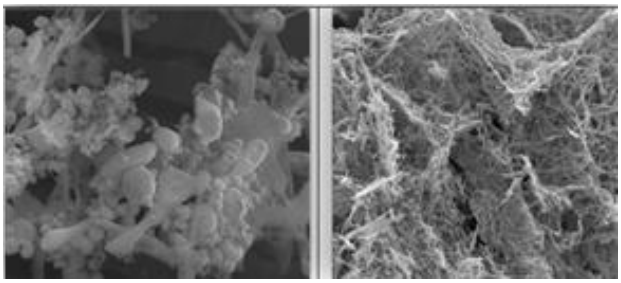

FIG. 1. SEM images of thermosensitive polymeric carriers before and after encapsulation.

Conclusions
The main goal of the research was the development of an effective method of the encapsulation of the model drug using thermosensitive polymer carrier. The conditions of encapsulation were selected and its efficiency was determined, which allowed to assess the percentage of the introduced salicylic acid. In addition, various studies were carried out using the following research techniques: SEM, DLS, UV-Vis and FT-IR, which allowed to analyze both the carrier and the carrier-drug system.

Acknowledgments
This research was supported by The National Centre for Research and Development — project LIDER/41/0146/L-9/17/NCBR/2018.

References
[1] A. Anju et al., *Acta Pharmaceutica Sinica B*, 2014, 4, 120-127.
[2] D. Schmaljohann, *Advanced Drug Delivery Reviews*, 2006, 58, 1655-1670.
[3] M.R. Aguilar, J.S. Román *Smart polymers and their applications* Burlington: Woodhead Publishing, 2014, 327-348.
[4] R. Hoare, D.S. Kohane, *Polymer*, 2008, 49, 1993-2007.
[5] M. Karimi et al., ACS *Appl Mater Interfaces*, 2016, 24, 8(33), 21107–21133.

UNDERSTANDING OF FLUOROURACIL NANOPARTICLES SONOCHEMICAL FORMATION FOR ITS CONTROLLED DELIVERY FROM POLYMERIC SURFACES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 36]

Introduction

The advancement in biomaterial design and engineering has led to the rapid development of novel polymeric materials with increasing complexity and functions. Controlled drug delivery systems (DDS), which are aimed to deliver drugs at predetermined rates and predefined periods, have attracted increasing attention. The main aspects which have to be considered while designing the system for regional drug delivery are the choice of drug molecules, which are suitable for the targeted tissue and the method of its anchoring to the medical device surfaces. Too weak binding results in burst effect and fast release of the therapeutic agent, while too strong one limits or even block the elution and as a result the therapeutic effect.

The direct insertion of bioactive molecules into the surface of the biomedical device is a promising strategy. Among several methods, which can be used to fabricate drug delivery systems, 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 solution over the polymeric surface, the antibiotic NPs are subsequently embedded into the exposed surface in a one-step process. This strategy allows for obtaining a composite NPs/polymer with prolonged drug release from the surface.

The formation of NPs increases the activity and bioavailability of the drugs, moreover, the tissue penetration by the drug can be significantly improved. The detailed understanding of the sonochemical formation of nanoparticles is still insufficient. Hence, in this work we propose the application of Molecular Dynamics simulations (MD) to give insights in the sonochemical formation of nanoparticles. Therefore, we investigated experimentally and by molecular dynamics simulations the first steps of fluorouracil nanoparticles formation via the sonochemical method.

Materials and Methods

Fluorouracil nanoparticles were formed and deposited on the oxygen plasma modified polymers (parylene C) using a homogenizer (Sonics Vibracell CV18) with a frequency of 20 kHz, amplitude 35%, and time 4 min. The size of the sonochemically formed fluorouracil NPs was determined using the 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. The developed system was thoroughly characterized in terms of particle size (NTA, TEM), surface (ATR-IR), and drug release kinetics (UV-Vis).

Atomistic molecular dynamics simulations were performed to investigate the early stages of fluorouracil nanoparticles formation. The atomistic MD simulations were carried out in an NVT ensemble using GROMACS 5.1.x software and the parameters for fluorouracil molecule were taken from the Amber03 force field.

Drug release studies of the parylene C with sonochemically embedded fluorouracil nanoparticles were performed in phosphate buffered saline solution (PBS) at 37°C.

Results and Discussion

The developed system was thoroughly characterized, before and after embedment, by spectroscopic and microscopic methods. It was revealed that the optimization of the applied ultrasound conditions resulted in the formation of nanoparticles (80-100 nm, FIG. 1A), while the molecular structure of the drug was preserved (confirmed by the FTIR spectra). The experiments reveled the possibility of embedding NPs into polymeric surface with the use of ultrasounds. In parallel using MD simulations the mechanism of fluorouracil nanoparticles nucleation was investigated. The aggregation of drug molecules at the bubble interface, which can be considered as and an early-stage of NPs formation, is shown in FIG. 1B. MD simulations provided valuable information concerning the fluorouracil solution composition (water/alcohol) for the effective formation of nanoparticles.

Drug elution studies were performed to determine stability of the fluorouracil NPs deposited on the polymeric surface.

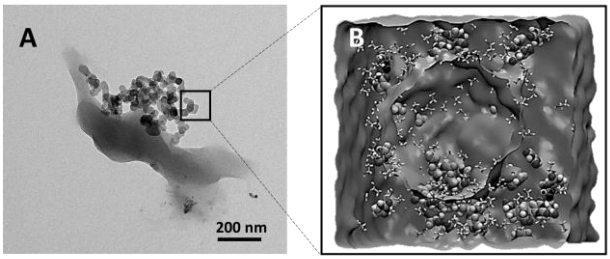

FIG. 1. TEM image of sonochemically created fluorouracil nanoparticles deposited on holey carbon mesh (A). MD simulation snapshot of fluorouracil aggregation at the bubble-solvent boundary (B).

Conclusions

Sonochemical synthesis is a powerful method for the production of nanostructured materials made of biologically active substances. The apparent benefits of the proposed sonochemical approach such as short preparation time, direct drug accessibility, lack of chemical wastes are pointed out. The molecular dynamics simulations provide the insights into the formation of drug nanoparticles and support the design of the synthesis protocol by adjusting the composition of the native solution.

References

[1] M. Gołda-Cępa, et al. Nanomedicine:NBM 14 (2018) 941-950. (2018)
[2] O. Grinberg, et al., J. Mater. Chem. B 3 (2015) 59-64.

FUNCTIONALIZATION OF POLYURETHANE SURFACES FOR IMPROVED BIOCOMPATIBILITY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 37]

Introduction

Esophageal cancer is at the forefront of the most commonly diagnosed cancers, and the sixth main cause of cancer–related mortality. The polyurethane membranes of esophageal stents, that prevent tumor overgrowth, should be stable and ensure reliable support against dysphagia, although, in long-term use, a significant loss of biostability is observed. Thus, the functionalizations of polyurethanes are necessary individually for the inner and outer sides of the stent. In order to obtain cover's best performance and stent durability, the outer side, constantly in contact with esophageal epithelium, has to be biocompatible while the inner side to prevent the clogging of the medical nutrients has to be antifouling. Such dual functionalization can be obtained with the use of plasma treatment by the introduction of surface functional groups. The superiority of the proposed method is based on its simplicity, efficiency, and environmental friendliness. Such an approach opens the doors to the development of polymeric biomaterials with a novel class of functionalized surfaces to help patients' palliative treatment and increase their quality of life.

A key starting point for designing cell–adhesive polymeric materials is to activate surfaces with functionalities that promote the adhesion and survival of selected cell types involved in the wound healing or tissue regenerative process. The proposed approaches for improvement of biomaterials include enhancement of adsorption of specific proteins and material modification by immobilization of cell recognition motives to control interaction between cells and polymeric substrates. The RGD sequence ((R: arginine; G: glycine; D: aspartic acid; Arg-Gly-Asp) is the most effective cell-recognition motif and has been used to stimulate cell adhesion on synthetic surfaces.

In this study we focus on exploring two different ways of surface functionalization of the polyurethane which are used as a cover of self-expandable nitinol esophageal stent.

Materials and Methods

The commercially available polyurethane samples provided by American Polyfilm, Inc were modified using oxygen plasma using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany) at pressure of 0.2 mbar. The varied parameters were the time of exposure to the plasma, which was in the range of 6 s to 10 minutes and the plasma generator power with the range of 50 to 100 W.

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 determination of contact angles and surface tension.

Changes in surface morphology of the polyurethane films were observed with the scanning electron microscope (Hitachi S-4700).

Results and Discussion

Plasma parameters, such as applied power, and exposition time have to be carefully adjusted based on the specific polymer and its application. Detailed characterization of the oxygen plasma treated surfaces is crucial, because the biological moieties are rigorously sensitive to the geometrical and/or chemical modifications of material.

The introduction of oxygen functional groups has a significant influence on hydrophilic properties of the examined material. Such modifications were followed by contact angle measurements. The changes in surface properties are represented directly by water contact angle. The unmodified polyurethane surface, with the Θ_{H2O} = 105° ± 3°, are hydrophobic. The water contact angles for the samples treated with oxygen plasma systematically decrease, for 30 s Θ_{H2O} = 60° ± 3°, for 1 minute Θ_{H2O} = 50° ± 2° and for 10 minutes Θ_{H2O} = 35°±3°. The observed changes in polyurethane surface induced by oxygen plasma treatment are significant in terms of its biocompatibility.

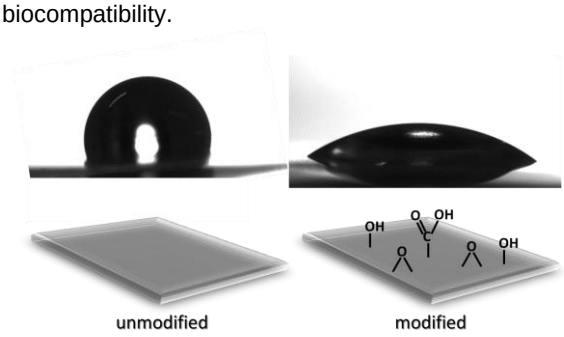

FIG. 1. Water contact angle measurement for unmodified polyurethane surface and for 1 min oxygen plasma modified (the introduced oxygen functional groups were spectroscopically identified).

Conclusions

The oxygen plasma modification on polyurethane surfaces, have a significant impact on the biocompatibility in terms of increased hydrophilicity. Since the assumptions for the functionalization procedure are of a general nature, the obtained results can be easily extended for other plasma feed gases and polymeric materials.

Acknowledgments

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SCIENTIFIC FUNDAMENTALS FOR ANTIBACTERIAL SURFACES DESIGN AND PREPARATION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 38]

Introduction

The medical device industry has made enormous progress during the past decades, owing to the gained knowledge on the development of advanced materials technologies. Among a wide range of biomaterials (e.g. metal, ceramic, polymer, carbon-based) graphenic surfaces are of great interest due to their specific advantages. These include large surface area, good mechanical properties, and electrical conductivity, as well as tunable surface functionalities which play a pivotal role in regenerative medicine applications [1,2]. Graphene-based biomaterials are extensively investigated in recent years in the context of biological and medical applications such as stem cells differentiation, tissue engineering, bone regeneration, dentistry, drug delivery, photothermal therapy, and bio-imaging [3,4]. Although several questions remain open, graphene family nanomaterials are an exciting promise in biomedical applications. Certainly, further investigations of the interactions of cells with graphenic surfaces, signalling pathways, biocompatibility as well as bacteria colonization are needed. [5,6].

Materials and Methods

Materials topography, application of nanoparticles, and introduction of specific functional groups are considered among various strategies of designing antibacterial surfaces. In this work, we have investigated the effect of oxygen plasma treatment of graphenic surfaces and the introduction of functional groups on changes in work function, wettability, surface free energy, and bacterial adhesion was checked. The plasma parameters were adjusted (generator power: <60 W, exposure time: <20 min) to limit the modifications to the surface without changing the bulk structure. The parent and modified graphenic surfaces were thoroughly characterized by RS, TG/DTA, SEM, XPS, contact angle, work function, and microbiological tests.

Results and Discussion

The effect of oxygen functional groups introduced to graphenic surfaces on bacterial adhesion was evaluated for a series of microorganisms: *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*. The modification of graphenic surfaces was accomplished by the application of low-temperature oxygen plasma treatment while adjusting the parameters for control surface modification (number of surface functional groups) without changing the bulk of the carbon materials. The introduction of surface dipoles ($C_{surf}^{\delta+}$-$OH^{\delta-}$) results in substantial changes not only in surface chemistry of graphenic sheets but also in biological response (bacterial adhesion). The obtained results clearly show that key factors of bacterial colonization are the electrodonor properties of the surface (work function) and the zeta potential of bacterial cells. The lowest colonization rate was observed for lower work function graphemic material (4.4 eV) and for bacteria with the lowest zeta potential (*E. coli*). The results were rationalized in terms of total interaction energy with the main contribution from electrostatic forces at the graphenic sheet-bacterial cell interface (FIG. 1).

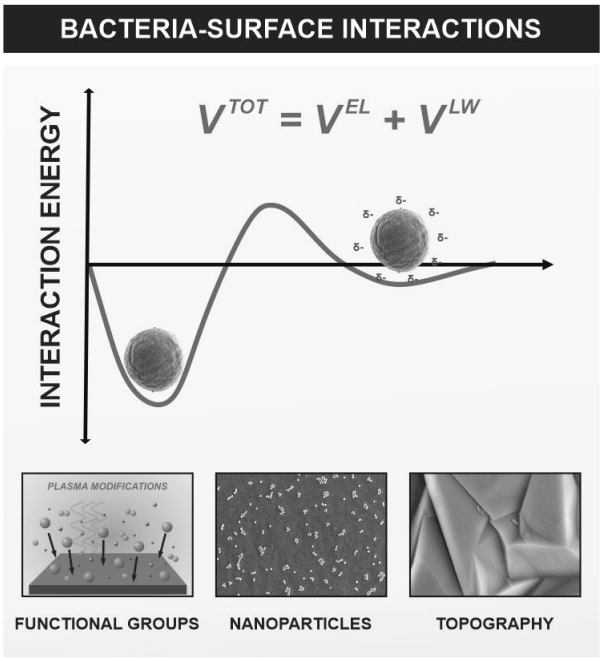

FIG. 1 Schematic energy profile for bacteria-graphenic surfaces interactions based on the DLVO theory. The integral approach of the surface electrodonor properties modification showing main influencing factors such as surface functional groups, nanoparticles, and topography.

Conclusions

It was found that even the short time of plasma modification results in a significant increase in work function, surface free energy and hydrophilicity of graphenic materials. The changes in bacteria charge and surface chemistry dramatically influence the bacterial adhesion. The bacterial colonization is facilitated by the low zeta potential. The results also pointed out the work function lowering of the graphenic biomaterial surface as an effective strategy for the infection risk limitation.

Acknowledgments

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References

[1] S. Kumar *et al.,* Mater. Sci. Eng. C. 106 (2020) 110154.
[2] S. Han *et al.*, Am. J. Transl. Res. 11 (2019) 3246-3260.
[3] M. Tahriri *et al.*, Mater. Sci. Eng. C. 102 (2019) 171-185.
[4] C. Nie *et al.*, Nano Todda. 26 (2019) 57–97.
[5] J. Ming *et al.*, ACS Appl. Bio Mater. 3 (2020) 704–712.
[6] S. Goenka *et al.*, J. Control. Release. 173 (2014) 75–88.

BACTERIA MEET NANOPARTICLES - ELECTRON MICROSCOPY INSIGHTS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 39]

Introduction

Nanoparticles (NPs) application in medicine has great potential for future advances not only in innovative therapies but also as a diagnostic tool [1]. Because of their biocompatibility and unique properties, such as surface plasmon resonance or superparamagnetism in some cases, NPs are widely investigated for cancer therapies, pharmacology, advanced diagnostics, treating bacterial infections, and antiseptics [2,3]. The last two are of special scientific interest, aiming at targeting pathogenic bacteria with the use of antibiotics alternatives [4]. However, understanding of NPs antibacterial interaction remains a great challenge. There are several possible mechanisms including mechanical damages of cells, oxidative stress, photo-killing, ions homeostasis disturbance, and proteins dysfunctions [5,6]. However, there is no doubt that independently on the mechanism involved, NPs need to be in direct contact with bacterial cells to achieve their antibacterial properties. Therefore, the initial stages of NPs bactericidal mechanisms are needed to be investigated to identify the principal parameters governing the NPs interaction with the bacteria [2]. This study addresses this issue via the application of advanced *in-situ* TEM observations.

Materials and Methods

Electron microscopy techniques allow for high-resolution observations but cannot be directly applied to biological samples. Typically, for TEM observations biological moieties have to be fixed during multi-step protocols. In this study a state-of-the-art, *in situ* liquid-phase transmission electron microscopy (LP-TEM) was applied to observe the interaction between bacteria and gold nanoparticles (15 nm) in real-time. The specialized Poseidon TEM holder and Protochips systems dedicated to in situ observations in liquids were used (FIG. 1). The reference strain used was *Staphylococcus carnosus* DSM 20501 (Deutsche Sammlung von Mikroorganismen und Zellkulturen), a typical gram-positive coccus, spherical in shape and forming grape-like clusters. The average size of a single cell ranges between 0.5 and 1.5 µm. The static observations were performed with an e-chip with a 5 µm spacer. The bacteria in water solution (~$6 \cdot 10^8$ /ml) were dropped on the large e-chip and subsequently dried. Next 0.1 µm of the AuNPs solution (~25 mg/l) was dropped in the small chip loaded into the holder. After the *in situ* observations the small e-chip was dried, loaded into a single tilt holder, and observed in BF and HAADF STEM mode.

Results and Discussion

For the in-depth studies of bacteria-NPs interactions, a dedicated in situ Poseidon TEM holder was successfully applied. The micrograph (FIG. 1) shows *ex post facto* observations of bacteria with adhered gold NPs. The investigations revealed that bacteria retain their properties despite being coated with gold nanoparticles. The bacterial cell life processes like cell division with the formation of the bacterial septum, intercellular nanotubes creation, and outer membrane vesicles formation were observed. In opposite to standard TEM observations, LP-TEM allows for direct investigations of fully hydrated and native cells in their natural liquid conditions. This approach provides new opportunities in life science microscopic investigations to study dynamic processes in real-time at a near-atomic scale.

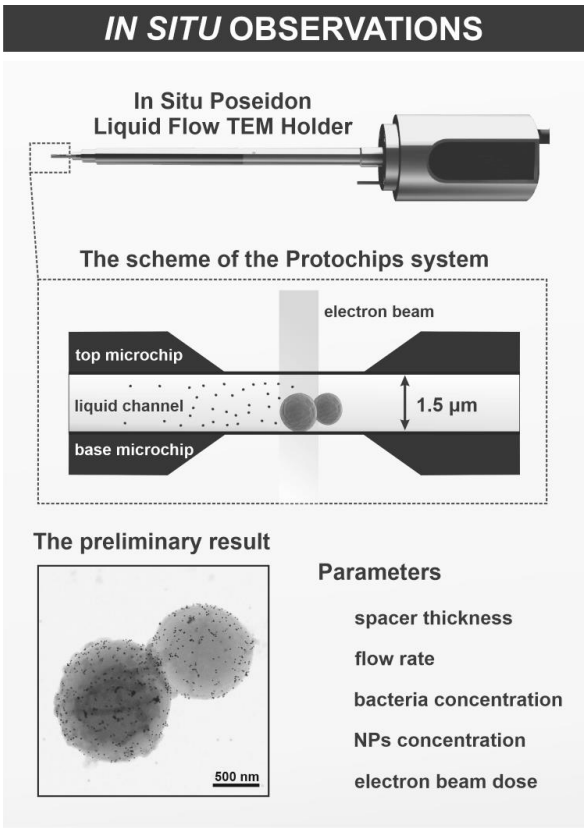

FIG. 1 Graphical representation of the approach of the *in situ* observations of bacteria-NPs interface showing dedicated Poseidon TEM holder, equipped with special Protochips system for electron microscopy observations in liquids and the obtain representative image of Au-coated *S. carnosus*.

Conclusions

Taking into account the novelty of application LP-TEM for biointerface studies allows for a unique insight into the bacteria-NPs interface. The studies also reveal that for effective observations, the methodology should be carefully optimized considering the size of a bacterial cell and chip size to provide suitable space within the liquid channel of the Protochips system as well as the incubation conditions.

Acknowledgments

W. Pajerski acknowledges the fellowship with the project POWR.03.02.00-00-I013/16

References

[1] Nabil, G. *et al.* Drug Discov. Today 24 (2019) 462-491
[2] Makvandi, P. *et al.* Adv. Funct. Mater. (2020) – *in Press*
[3] Cryer, A. M. *et al.* Pharmacol. Ther. 198 (2019) 189-205.
[4] O'Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, London, UK (2016).
[5] Kadiyala, U. *et al.* Curr. Pharm. Des. 24 (2018) 896-903.

HOW TO STIMULATE POLYMERIC SURFACES BIOCOMPATIBILITY AND HYDROXYAPATITE FORMATION: EXPERIMENTS SUPPORTED BY MD SIMULATIONS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 40]

Introduction

Polymers have been widely used for the last 3 decades in medical applications, such as coated transducers, neural prosthesis, catheters, and parts of orthopedic implants. Because of a plethora of existing possibilities, three questions need to be addressed in order to obtain the biomaterial tailored for the desired site in the body: (a) what is the required function? (b) Should functional groups be introduced? If yes, which one? (c) What is the most effective surface coverage?

For the biocompatibility of the adherent cells, such as osteoblasts, polymeric surfaces can be successfully transformed from hydrophobic to hydrophilic using oxygen plasma treatment. This modification results in the introduction of oxygen-containing functional groups (−OH, −CHO, −COOH). Such functionalities have been described to be crucial for initial steps of osteogenesis, which induces wound healing and consequently osseointegration

This study aimed to gain an in-depth molecular insight into the experimentally observed changes in surface wettability and nucleation of calcium phosphate at the functionalized polymer−body fluid interface. We employ the molecular dynamics simulations to reveal the role of surface functional groups formed during the oxygen plasma treatment in the biocompatibility and mechanism of calcium phosphate formation.

Materials and Methods

To modify the polymeric surfaces, oxygen plasma treatment was carried out using a Diener electronic Femto plasma system (Diener Electronic) at 50 W and an oxygen partial pressure of 0.2 mbar [1]. The samples were characterized with the use of spectroscopic (RS, IR, XPS), microscopic (fluorescence microscopy, SEM), and biological (microbiological, cell culture, in-vitro SBF incubation) methods. The atomistic MD simulations were performed according to our published models [2,3]. Four different functional groups, corresponding to different ways of surface modification, were considered, namely, −OH, −CHO, and $-COO^-$.

Results and Discussion

Upon oxygen plasma treatment, the originally hydrophobic polymeric surfaces (Θ_w=90º) turn hydrophilic with a dramatic decrease of water contact angle value to Θ_w=0.1º. As a consequence, significant differences were observed in the case of the calculated values of Surface Free Energy (SFE) as well as their corresponding polar (γ_s^p) and dispersive (γ_s^d) components. Initially, the SFE of unmodified polymer 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). Modification 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. 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. This founding has an important significance for the modified polymeric surface; the experimentally obtained ratio of dispersive and polar components γ_s^d/γ_s^p = 0.5 is in line with the theoretically determined 60% surface coverage for −OH, such that the corresponding ratio of dispersive and electrostatic energies ($E_{dispersive}/E_{electrostatic}$) is 0.56. Such optimized modification with oxygen-containing surface groups significantly enhances the interactions between body fluid ions and the polymeric surfaces, observed experimentally as calcium phosphate formation. The results were discussed in terms of MD simulations of the calcium phosphate clustering (FIG. 1).

Surface functional groups promote the clustering of calcium and phosphate ions in the following order: −OH > −CHO > −Cl (unmodified polymer) ≈ $-COO^-$. This promoting role of surface functional groups is explained as stimulating the number of Ca^{2+} and HPO_4^{2-} surface contacts as well as ion chemisorption.

Conclusions

In the study, the superiority of the −OH groups (50% coverage) was identified as the most effective sites for calcium phosphate nucleation. The advantage of the combined experimental and theoretical approach is pointed out as effective for biointerface design and fabrication.

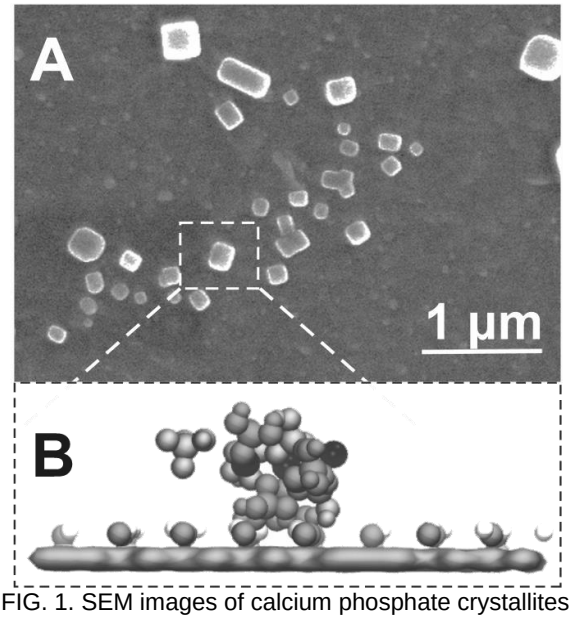

FIG. 1. SEM images of calcium phosphate crystallites formed on oxygen-plasma modified polymeric surface (A) and characteristic MD snapshot presenting the last stage of the calcium phosphate nucleation process (B).

Acknowledgments

This work was supported by the National Science Centre, Grant 2019/35/D/ST5/03107.

References

[1] M. Golda-Cepa *et al., ACS applied materials & interfaces*, 8, 22093-22105.
[2] M. Golda-Cepa *et al., ACS Applied Materials & Interfaces* 9 (2017): 16685-16693
[3] M. Golda-Cepa *et al., ACS Applied Materials & Interfaces* 12 (2020): 12426-12435

DESIGN AND TAILORING OF POLYMERIC BIOMATERIALS SURFACES: PREVENTION OF BIOMATERIALS-CENTERED INFECTIONS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 41]

Introduction

In the EU each year at least 500 000 patients undergo surgeries that required stabilizers and endoprostheses implantation and approx. 2% required revision surgery due to implant-related infections. Biomaterial-Centered Infection (BCI) may lead to secondary complications i.e. amputations, morbidity and even mortality. Indeed, infection treatment is associated with significant costs, approx. 6.5 times higher compared to patients without infection. Thus, the prevention of BCI is one of the most challenging issues in orthopedic and trauma surgery. Although several BCI preventive strategies have been developed i.e. the preoperative administration of antibiotics, standardized sterilization procedures with restricted, detailed protocols, still more than 25% of all hospital-acquired infections are medical devices-related. Therefore, the investigations on novel biomaterials should integrate biocompatibility and anti-infection functions and tuned them together, which is a real challenge for surface functionalization.

In the study different kinds of medical-grade polyurethanes materials are used, due to their versatility. These materials can be rigid, semirigid or flexible with excellent biocompatibility, outstanding hydrolytic stability, superior abrasion resistance, outstanding physical strength, and high flexure endurance. The availability of polyurethanes in several forms, i.e. of adhesives, coatings, sealants, rigid and flexible foams as well as textile fibers allow their use in pacemakers, catheters, vascular grafts, heart assist balloon pumps, artificial heart bladders and wound dressings.

The initial stage of the bacterial attachment process is governed by the interplay between the properties of the bacterium and solid surface, which is mediated by the body fluids ions, as summarized in FIG. 1. The ability of bacterial cells to adhere and form a biofilm is a crucial feature for their survival in complex environments (i.e. human body). As a result, microorganisms developed several strategies for specific and non-specific interactions with the surfaces. Despite enormous research efforts in the field of biomaterials, there is a lack of solutions preventing bacterial adhesion with excellent biocompatibility. The study aimed to optimize plasma treatment parameters of polyurethanes in such a way to obtain biocompatible and anti-infection surfaces.

Materials and Methods

The medical-grade polyurethane surfaces were plasma-functionalized with the use of different feed gases: air, O_2, CO_2, and N_2. The samples were characterized with the use of surface- (water contact angle measurements, XPS) and bulk- (XRD, TG/DTA, RS) dedicated methods. For the selected functionalized samples, the microbiological tests were performed (live/dead fluorescent staining, adhesion rate).

Results and Discussion

The plasma treatment allows to obtain the polyurethane surfaces with controlled surface functional groups coverage. The parameters of plasma modifications were precisely adjusted in such a way to limit the functionalization to the surface, without changing the bulk properties of the materials (monitored by changes in crystallinity of the polymers and melting point determination).

It was revealed that the introduced functional groups are beneficial for biocompatibility, however, they increase the risk of infection. The obtained polyurethane surfaces differ significantly in terms of surface free energy (SFE) 80 mJ/m^2-20 mJ/m^2. Such surfaces can be used for further studies, however, their optimal biomaterial application will be diverse. Polymeric surfaces with high SFE (>50 mJ/m^2) are suitable for bone tissue while those with low SFE (<50 mJ/m^2) can be used for blood contact.

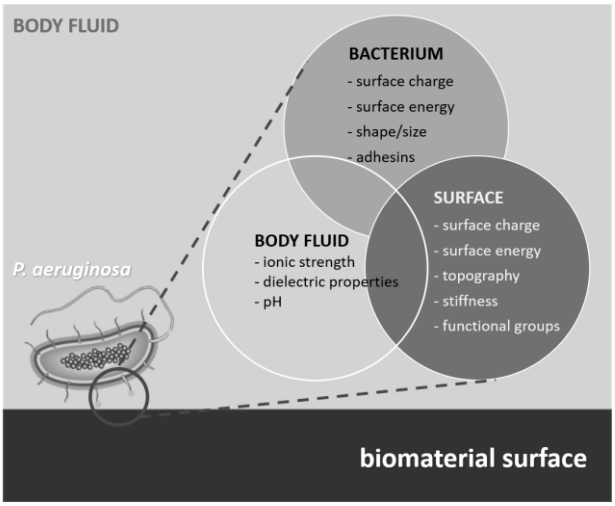

FIG. 1. A schematic representation of the main factors that influence initial bacterial attachment to abiotic surfaces.

Conclusions

Polyurethanes, as the most versatile polymeric family used as biomaterials, can be successfully surface modified by plasma. It is possible to obtain materials with dramatically different SFE and therefore, compatible with targeted tissues.

Acknowledgments

This work was supported by the National Science Centre, Grant 2019/35/D/ST5/03107.

References

[1] Shelke, N. B., *et al.* Natural and Synthetic Biomedical Polymers (Elsevier, 2014).

MODIFICATION OF CARBON MATERIALS SURFACES BY PLASMA: EFFECT OF THE INTRODUCED FUNCTIONAL GROUPS ON BIOCOMPATIBILITY AND ADHESION OF MICROORGANISMS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 42]

Introduction

Carbon is one of the most common and fascinating elements on the Earth, present mainly in the form of biomolecules and organic compounds as well as elemental carbon materials. Carbon exhibit the unique ability to form strong covalent bonds between atoms, which is related with various hybridization states: sp, sp^2, sp^3. Additionally, carbon structures are relatively easy to functionalize with surface and bulk heteroatoms. As a consequence, a wide range of carbon-based nanomaterials can be formed offering a broad range of structures' including 0 - 3D dimensionalities.

Carbon materials are chemically inert, they are stable in strongly acidic and basic solutions. Nowadays, a majority of the academic and industrial research efforts is focused on the tuning of these unique properties for numerous applications. Indeed, in recent years carbon materials have attracted much attention in the field of biotechnology, medicine, and biomedical engineering. The research includes antibacterial and antiviral surfaces, tissue engineering, drug delivery, biosensing, cancer targeting, photothermal therapy, and electrical stimulation of cells to mention a few.

Although the investigations are carried out intensively and extensively, there is still a knowledge gap in the understanding of the interactions at the interface of these materials and surrounding biological moieties. Owing to the broad spectrum of possible structures and mechanical properties carbon lends great versatility in designing of implant materials. Nevertheless, each newly developed carbon material requires a specific assessment and optimization of the key functions required for medical devices: stability, biocompatibility, anti-infection and therapeutic.

The aim of the study was the controlled introduction of biologically-relevant surface species with a precisely adjusted concentration into the carbon materials surfaces: CNTs, graphenic sheets, carbon spheres. In the next step, we explored the correlation between the surface chemistry and biological response.

Materials and Methods

To modify the carbon-based materials, plasma treatment was carried out (Diener electronic Femto plasma system). The samples were characterized with the use of spectroscopic (RS, IR, XPS, SIMS-TOF, LDI-TOF-MS), microscopic (SEM, TEM) and microbiological (live/dead, adhesion rate) methods. The work function values (Φ) of the carbon materials, the contact potential difference (V_{CPD}) measurements were performed with KP6500 probe. The experimental results were supported by molecular modelling (DFT), according to our published computational models [1]. Four oxygen functional groups, corresponding to different ways of surface modification, were considered, namely, −OH, −CHO, −COOH, and =O.

Results and Discussion

The plasma parameters (power, pressure, time) have been optimized and the treatment was confined to the surface region changing its key parameters such as electronic properties (work function increase from 4.4 eV to 6.1 eV) and wettability (water contact angle decreased from 94° to 7°) while preserving the bulk structure. For quantification of the number of introduced functional groups, a combined experimental and theoretical approach was applied. The Helmholtz relation between measured work function values and calculated dipole moment of surface oxygen groups was successfully used. The examples of calculations results showing the formed surface dipoles with the imposed electrical isosurface are presented in FIG. 1.

Besides, a straightforward correlation between Gram-positive bacteria adhesion (*Staphylococcus aureus*) to the carbon-based surfaces, and their electrodonor properties (work function) was discovered. A similar effect was observed for Gram-negative bacteria strain (*Pseudomonas aerugionosa*). The bacteria coverage systematically increased (by a factor of three) with the plasma treatment from 3.2% (untreated) to 9.2% (oxygen plasma modified) [2]. Thus, it may be concluded that the colonization of graphenic surfaces strongly depends on the specific characteristics of the surface (oxygen concentration, wettability, electronic properties).

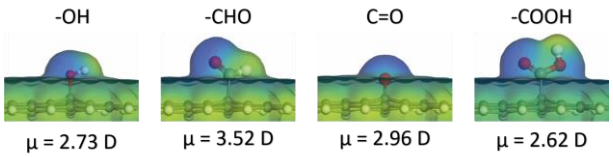

FIG. 1. The oxygen-containing groups on the graphenic sheets together with the electrostatic potential mapped on the electron density isosurface and calculated dipole moments (DFT, Dmol3, hybrid B3LYP).

Conclusions

The concentration of surface functional groups is a key factor in biocompatibility and bacterial adhesion to carbon-based materials surfaces. The plasma treatment can be successfully used as the modification method of these materials, however, the operating parameters have to be adjusted individually for each type of material. A straightforward correlation between bacteria adhesion to the carbon-based surfaces and their electrodonor properties was discovered. The obtained results provide the practical guidelines for the design and development of carbon-based nanomaterials.

References

[1] Duch, Joanna, et al. "Work function modifications of graphite surface via oxygen plasma treatment." Applied Surface Science 419 (2017): 439-446.

[2] Pajerski, W., et al. "Bacterial attachment to oxygen-functionalized graphenic surfaces." Materials Science and Engineering: C (2020): 110972.

CHARACTERIZATION OF PARTIALLY COVERED SELF-EXPANDABLE METALLIC STENTS FOR ESOPHAGEAL CANCER TREATMENT: IN VIVO DEGRADATION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 43]

Introduction

Squamous cell carcinoma of the esophagus is the fourth cause of death in males and seventeenth in females. There is no change or a slight decrease in incidence over the last three decades [1]. More than 50% of patients present with an unresectable tumor, progressive weight loss and dysphagia, require palliative treatment. Among the many available methods of palliation, 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 relatively 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 tumor ingrowth, but overgrowth beyond ends of the stent and granulation tissue formation remains an issue.
The study aimed to investigate the impact of long-term usage in the body on the physicochemical properties of the partially covered esophageal stents.

Materials and Methods

For the investigations, 16 partially-covered self-expandable metallic stents (SEMS) 7–12 cm long and a diameter of 18 mm (Ultraflex Boston Scientific, USA) were used. For the physicochemical investigation as the obtained stents were cut into 1x1 cm coupons. The morphology of the NiTi stent and polyurethane covered surfaces were evaluated by SEM. The properties of polymeric samples were analyzed using DSC. The measurements were carried out in a temperature range of 25–600°C with a heating rate of 10°C min^{-1} in Ar flow of 50 cm^3 min^{-1} [2]. The changes within the surface of polyurethane were followed by contact angle measurements (CA) [3]. ATR-FTIR analyses of the polymeric films were performed in order to analyze the structural changes of polyurethane, the spectra were recorded in the range 4000–650 cm−1. DMTA was used to determine the glass transition temperature (Tg), taken as the maximum of tan δ and the maximum of loss modulus, E''. The relaxation spectrum was scanned from –70 to 150°C, at a frequency of 1 Hz, and a heating rate of 3°C/min

Results and Discussion

Partially covered self-expandable metallic esophageal stent (SEMS) placement is the most frequently applied palliative treatment in esophageal cancer. Structural characterization of explanted 16 Nitinol-polyurethane SEMS (the group of 3 females, 13 males, age 40-80) was performed after their removal due to dysfunction. The adverse bulk changes in the polymer structure were visualized with the SEM (FIG. 1), analyzed with the use of TGA, DMTA, and ATR-IR and discussed in terms of melting point shift (9ºC), glass transition shift (4ºC), differences in viscoelastic behavior and systematic decrease of peaks intensities corresponding to C–H, C=O, and C–N polyurethane structural bonds. The scanning electron microscopy observations revealed all major types of surface degradation, i.e. surface cracks, peeling off the polymer material, and surface etching. The changes in the hydrophobic polyurethane surfaces were also revealed by a significant decrease in wettability (74º) and the corresponding increase of the surface free energy (31 mJ/m^2). It is worth emphasizing that substantial differences were observed between the proximal (esophagus) and distal ends (stomach) of the stents which were discussed in terms of interaction with specific body fluids.

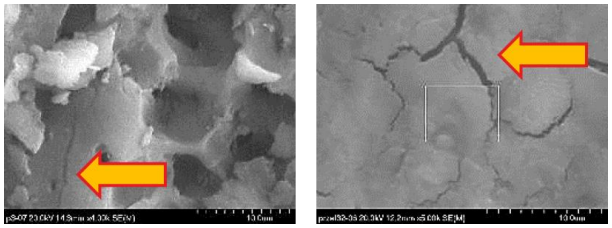

FIG. 1. The cracks (marked by arrows) in polyurethane surface resulting from exposure to the human body environment revealed by SEM images.

Conclusions

The obtained results also show that the contact angle and the polymer melting temperature can be considered as suitable parameters for analyzing the extent of the stent degradation processes. To our best knowledge, this work is the only report in the literature that shows the influence of chemo- and radiotherapy and the role of the microenvironment of the esophagus and stomach on the structure of the polyurethane/nitinol stents.

References

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

CHITOSAN HYDROGELS MODIFIED WITH SILK, GRAPHENE OXIDE OR REDUCED GRAPHENE OXIDE

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Introduction

The aim of the work was to produce and characterize nanocomposite hydrogels based on chitosan, modified with silk powder and carbon nano-additives in the form of graphene oxide or reduced graphene oxide.

The variety of favourable properties of chitosan, such as biocompatibility, biodegradability, antibacterial or antioxidant activity make this polysaccharide a polymer of choice in many medical applications e.g. in tissue engineering, wound healing, and drug delivery systems [1]. Also, silk is increasingly being used in medicine because it consists fibroin which improves collagen deposition and fibroblast proliferation [2]. In recent years, a “cutting edge materials” used in variety fields and applications have been graphene-family nanoparticles. Their unique two-dimensional planar structure vests exceptional properties e.g. super conductivity, chemical and mechanical stability, large surface area and biocompatibility. Graphene and graphene oxides modified composites have a huge potential for regenerative medicine of all types of tissues including nerves, bones, cartilages, skeletal and cardiac muscles, skin and adipose tissue regeneration [3]. Such composites might be used also in gene and small molecular drug delivery, for biofunctionalization of protein, in anticancer therapy, or as an antimicrobial agent carriers for bone and teeth implantation.

Materials and Methods

In this study a natural polymer matrix – hydrogel based on combination of chitosan (CS) and silk (SP) – was reinforced with two types of carbon nanoparticles: graphene oxide (GO) and reduced graphene oxide (rGO) (ITME, Poland) with various weight content.

To obtain composite “a solution-evaporation casting method” was applied and as a result thin foils were acquired. To prepare liquid matrix, a chitosan powder mixed with a silk powder in 4:1 ratio was dissolved in lactic acid. Then, matrix modifiers were introduced in following amounts: 0.5, 1.5, 3wt%. One group of samples was modified with GO and the second one with rGO. Each type of mixture was transferred into a Petri dish and left overnight to dry. When ready, foils were physically crosslinked by neutralization in 1M sodium hydroxide solution. In the end, six types of composites were obtained: CS/SP/0.5GO, CS/SP/1.5GO, CS/SP/ 3GO, CS/SP/0.5rGO, CS/SP/1.5rGO, CS/SP/3rGO. For reference purpose, foils made of pure CS and CS/SP were prepared in the same manner. After rinsing in water, the samples were incubated in distilled water and SBF. Morphology of the materials was examined before and after incubation. The impact of the type and amount of the introduced nanoadditives and the incubation process on the properties of the hydrogels was assessed.

Results and Discussion

Microscopic observations revealed that depending on the composition different morphology was obtained. For CS and CS/SP, crosslinking process had no effect on the flatness and smoothness of the samples surface, whereas for composites modified with GO and rGO high surface development with a mesh of tiny tubular channels was observed. The higher content of GO or rGO, the more distinct effect was present. In all types of tested materials ten-week incubation in water did not change surface characteristics. Sample images of CS, CS/SP, CS/SP/3GO and CS/SP/3rGO after incubation are shown in FIG. 1.

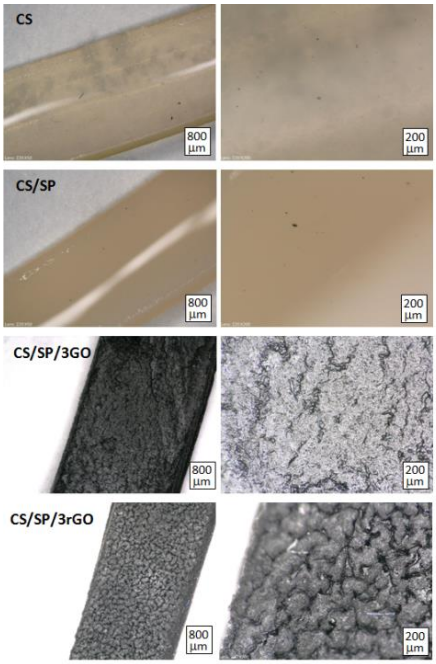

FIG. 1. Microscope images of hydrogels: CS, CS/SP, CS/SP/3GO and CS/SP/3rGO after 10-week incubation in distilled water; (Keyence VHX900, mag. 50x and 200x).

Bioactivity of tested materials was verified in SBF. On flat surface of CS foil there were only few spots of mineralized apatite. Addition of silk (CS/SP) improved this effect a bit, but the best results were observed for GO and rGO modified ones, were many apatite-like structures were observed. The amount of the modifier was irrelevant.

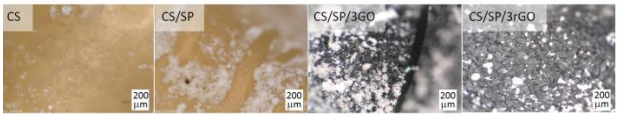

FIG. 2. Mineralization of apatites on different types of foils after 10 weeks incubation in SBF.

This leads to the conclusion that these materials have high bioactivity and potential to use in bone or cartilage regeneration. Such a statement cannot be placed for not modified chitosan.

References

[1] P. Domalik-Pyzik, et al. Chitosan-Based Hydrogels: Preparation, Properties, and Applications. In: Mondal M. (eds) Cellulose-Based Superabsorbent Hydrogels. Polymers and Polymeric Composites. Springer, Cham. 2019

[2] M.H. Kim, et al., Silk fibroin/hydroxyapatite composite hydrogel induced by gamma-ray irradiation for bone tissue engineering. Biomaterials Research 21, 2017, 1-9

[3] Priyadarsini, S.,et al. Graphene and graphene oxide as nanomaterials for medicine and biology application. J Nanostruct Chem 8, 2018, 123–137

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MECHANICAL PROPERTIES OF HYDROGELS IN A CHITOSAN/ SILK/GRAPHENE SYSTEM

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[ENGINEERING OF BIOMATERIALS 158 (2020) 45]

Introduction

Since 1960s, hydrogels have been intensively studied and developed. Their high water content is a big advantage because it promotes biocompatibility but at the same time, their mechanical properties are very low. Thus, during surgical procedure, such implants are very prone to damage. Recent research are focused on improving their mechanical properties and vesting additional functions. One of the most promising paths of modification is to incorporate nanoparticles into hydrogel matrix. Nanoforms of carbon, e.g. nanotubes, graphene, graphene oxide, seem to be excellent choice for this purpose. Such a "hydrogel–carbon nanoparticles" combination creates multifunctional material that has a potential to be used in regenerative medicine [1-3].

Materials and Methods

In this study a natural polymer matrix – hydrogel based on combination of chitosan (CS) and silk (SP) – was reinforced with two types of carbon nanoparticles: graphene oxide (GO) and reduced graphene oxide (rGO) (ITME, Poland) with various weight content.

To obtain composite "a solution-evaporation casting method" was applied and as a result thin foils were acquired. To prepare liquid matrix, a chitosan powder mixed with a silk powder in 4:1 ratio was dissolved in lactic acid. Then, matrix modifiers were introduced in following amounts: 0.5, 1.5, 3wt%. One group of samples was modified with GO and the second one with rGO. Each type of mixture was transferred into a Petri dish and left overnight to dry. When ready, foils were physically crosslinked by neutralization in 1M sodium hydroxide solution. After rinsing in water, the samples were cut into 4mm width strips. In the end six types of composites were obtained: CS/SP/0.5GO, CS/SP/1.5GO, CS/SP/3GO, CS/SP/0.5rGO, CS/SP/1.5rGO, CS/SP/3rGO. For reference purposes, foils made of pure CS and CS/SP were prepared in the same manner. To examine their mechanical properties, static tensile tests and analyses of strain-stress curve were performed (Zwick 1435). Tensile strength R_m, Young modulus E, maximum deformation ε_{Fmax} were characterised.

Results and Discussion

Mechanical testing results were collected and compiled as shown in graphs (Fig.1-3). As all materials are hydrogels it is not surprising that obtained results are rather low. Reference samples made of CS and CS/SP have tensile strength of 1,6 MPa and 1,2 MPa, respectively. Young's modulus values for CS/SP (4,0 MPa) were higher than for CS (3,6 MPa). When results of composites materials were analysed, it has been proven that in all cases except one (CS/SP 0.5rGO), addition of nanoparticles weakens a material and obtained composites have poorer mechanical properties than matrix itself (CS, CS/SP). The values of tensile strength for all composites foils were congruent and varied from 0,5 MPa to 0,8 MPa. Young's modulus decrease in range of 20-30% was observed. In general, it can be stated that from mechanical point of view introducing nanoforms of carbon into chitosan and chitosan/silk matrix incurred negative effects. Compared to reference, composite samples were twice weaker and their ranges of deformation were also reduced. These effects might be a result of poor distribution of nanoparticles in a volume of the matrix, thus nanoparticles weakened the material instead of strengthening it.

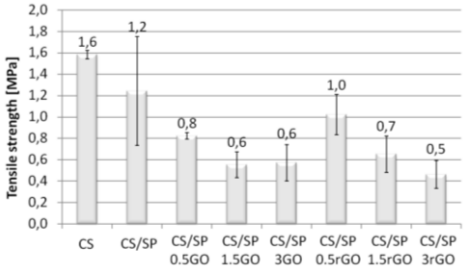

FIG. 1. Tensile strength of obtained hydrogels.

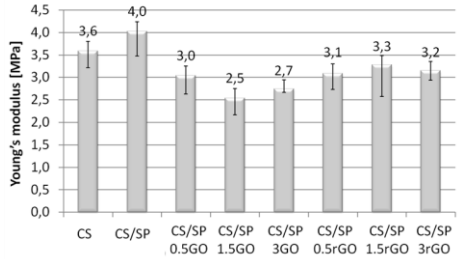

FIG. 2. Young's modulus of obtained hydrogels.

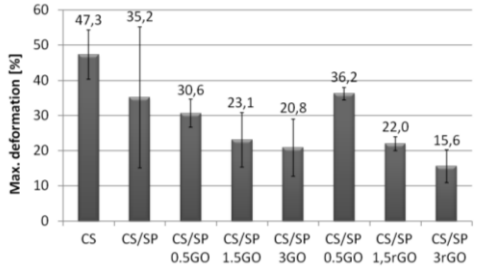

FIG. 3. Maximum deformation of obtained hydrogels.

Conclusions

The type (GO or rGO) and the quantity of carbon nanoparticles addition (0,5-1,5 wt%) have influence on material's tensile strength and deformation. Nevertheless, Young's modulus was not so susceptible and didn't change as much, depending on material composition. It is suspected that no chemical bonding between additive and matrix was created. Based on the results, it can be stated that in the case of modifying CS and CS/SP matrix rGO is a better choice than GO. Although it has to be in mind that these types of nanoadditives have also an impact on other properties of the material, e.g. biological and that should be investigated in a following study.

Acknowledgments

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References

[1] K. Sikorska, P. Rico, M.Salmerón-Sánchez, B. Szaraniec, Development of functional hydrogels for controlled ion release Engineering of Biomaterials 153, 2019, 99.
[2] M. L. Oyen, Mechanical characterisation of hydrogel materials; International Materials Reviews 59, 2014, 44–59
[3] N. Das, Preparation methods and properties of hydrogel: a review; International Journal of Pharmacy and Pharmaceutical Sciences 5, 2013, 112–117

MORPHOLOGICAL AND BIOCHEMICAL FEATURES OF MICROORGANISMS INHABITING THE SURFACES OF PERSONALIZED FEMORAL IMPLANTS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 46]

Introduction

Estimation and characterization of bioburden on healthcare products is a crucial step in the determination of sterilization parameters [1]. Evaluation of this parameter is also necessary to set up the contamination control program for a sterilization procedure [2]. A thorough knowledge of the physiological and biochemical characteristics of the microorganisms, colonizing the surfaces of medical implants, allows adjusting the method and dose of the sterilizing agent [3]. The study presents the results of the assessment of the biodiversity of microorganisms, colonizing the prototypes of femur implants, immediately after their manufacturing.

Materials and Methods

Five femoral implants were used in the research. Before the analysis, the implants were cleaned from post-production contamination. The number of facultative non-fastidious, aerobic, spore-forming and anaerobic bacteria, as well as yeasts and moulds, were determined by membrane filtration, according to PN-EN: ISO 11727-1 and Polish Pharmacopoeia, edition X. The morphology of isolated bacteria was observed under the light microscope (BX63, Olympus, Tokyo, Japan) after Gram staining. The biochemical features were identified using Api®ZYM tests (BioMerieux, Marcy-l'Étoile, France), as well as Bactident® Oxidase (Merck, Darmstadt, Germany) and Bactident® Aminopeptidase (Merck, Darmstadt, Germany) following the manufacturer's instructions.

Results and Discussion

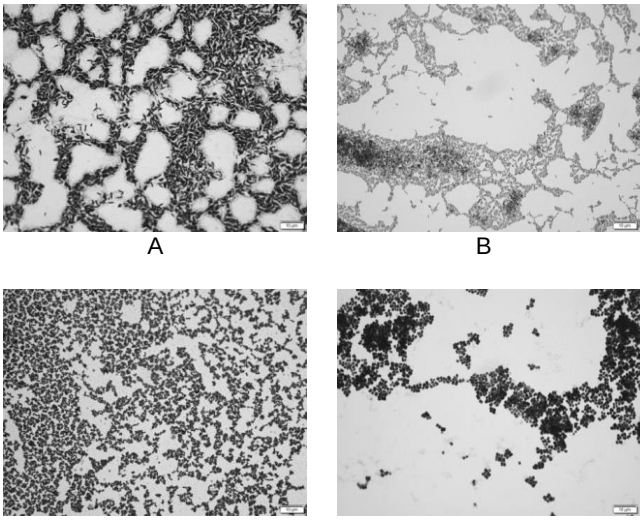
The surfaces of the implants were contaminated by bacterial strains. Yeast and moulds were not detected (TABLE 1).

TABLE 1 Determination of bioburden

№	Number of facultative, non-fastidious, aerobic bacteria	Number of anaerobic bacteria	Number of spore-forming bacteria	Number of yeasts and moulds
	cfu/implant			
1	nd	2	1	nd
2	4	nd	nd	nd
3	2	nd	nd	nd
4	nd	1	nd	nd
5	nd	nd	nd	nd

nd – not detected

Facultative and aerobic bacteria were the dominant microflora. However, anaerobic and spore-forming bacteria were also present. Their morphologies were described based on microscopic images (FIG. 1).



A B C D

FIG. 1 An exemplary morphology of isolated bacteria, Gram-positive bacilli (A), Gram-negative rod shaped (B), Gram-positive cocci (C), Gram-negative cocci (D)

Most of the isolated strains were Gram-positive, cocci. We also identified Gram-positive bacilli, Gram-negative cocci and Gram-negative rod-shaped bacteria. Despite the morphological similarities of the Gram-positive cocci, the isolated strains showed slightly different biochemical characteristics. The differences concerned mainly alkaline phosphatase, leucine arylamidase and β-glucosidase. Large variations of enzymatic activities were also demonstrated by Gram-positive bacilli. Inequalities were related to oxidase, valine arylamidase, α-galactosidase, β-galactosidase and β-glucosidase.

Conclusions

The bacteria were dominant microflora colonizing the surfaces of the implants. Although the number of tested microorganisms was relatively small, the biochemical and morphological characteristics showed significant diversity. A cursory analysis of bioburden can increase the risk of incorrect adjustment of sterilization conditions and thus, the risk of patient infection.

Acknowledgements

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References

[1] H. Atchia, Guide to Microbiological Control in Pharmaceuticals and Medical Devices, CRC Press (2006) 111-120
[2] S. Moondra, N. Raval *et al*., Dosage Form Design Parameters. Academic Press (2018) 467-519.
[3] S. Govindaraj, M.S. Muthuraman, Int J ChemTech Res 8 (2015) 897-911.

IDENTIFICATION AND CHARACTERIZATION OF MICROBIOLOGICAL CONTAMINATION SOURCES IN THE ENVIRONMENT OF PRODUCTION OF PERSONALIZED FEMORAL IMPLANT

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[ENGINEERING OF BIOMATERIALS 158 (2020) 47]

Introduction

Microbiological contamination is a serious problem in medical implants manufacturing [1]. The high number of microorganisms on the surface of implant can lead to ineffective sterilization process and, as a consequence, to patient infection [2]. The first and fundamental step to reduce the risk of contamination is identification of its sources. The study shows changes in the number of microorganisms, at critical control points, in subsequent seasons, in the femoral implant manufacturing environment.

Materials and Methods

The researches were carried out in a Polish tools factory. The main microbiological contamination sites were: emulsifying oils used during material processing, surfaces around the production stations and the air in the production hall. We determined the total number of mesophilic bacteria, as well as yeasts and moulds. Additionally, in the case of emulsifying oils and surfaces, the number of spore-forming and anaerobic bacteria was count. Airborne microbiological contamination was evaluated using the Koch sedimentation method. Other environments were examined using the swab and pour plating method. TSA medium was used for determination of the number of mesophilic and spore-forming bacteria. The anaerobic bacteria were isolated on Schaedler's agar, whereas yeasts and moulds on Sabouraud's agar with chloramphenicol.

Results and Discussion

Regardless of the season, the total number of bacteria in the air was around 10^5 cfu/m^3 (FIG. 1A). The number of yeasts and moulds were varied significantly. Maximum values were detected in spring and summer (FIG. 1B). Nevertheless, microbiological air pollution was high.

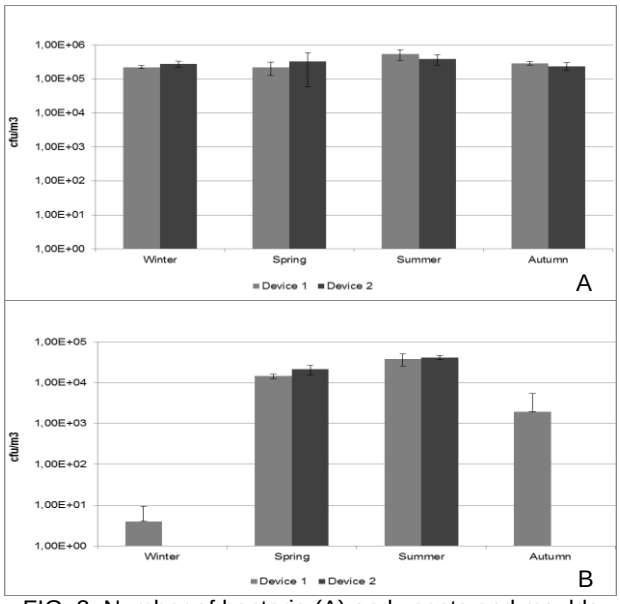

FIG. 2. Number of bacteria (A) and yeasts and moulds (B) in the air.

The main source of microbiological contamination was the emulsifying oil used in the device 2. The total number of aerobic bacteria reached 10^7 cfu/ml, the other groups of microorganisms were also presented in high number. Whereas, in the emulsifying oil used in device 1, microorganisms were usually not detected. This had an impact on microbiological contamination inside the devices used for implants manufacturing (TABLE 1). The total number of microorganisms in device 2 reached even 10^4 cfu/cm^2.

TABLE 2 Microbiological contamination inside and outside of the devices

	Total number [cfu/cm^2]	
	Inside	Outside
Device 1		
Mesophilic bacteria	8,76±8,73	(9,50±9,20)·10^1
Anaerobic bacteria	<1	<1
Spore forming bacteria	<1	<1
Yeasts and moulds	1,51±1,50	6,00±4,90
Device 2		
Mesophilic bacteria	(5,76±3,15)·10^4	(1,45±0,77)·10^1
Anaerobic bacteria	(3,43±3,10)·10^4	<1
Spore forming bacteria	(8,55±5,77)·10^1	<1
Yeasts and moulds	(8,79±6,09)·10^2	4,50±4,43

Conclusions

Evaluation of microbiological contamination is necessary to develop the control program which aims to reduce the risk of lack of sterility of medical implants. Determining the source and level of impurities is necessary to choose the appropriate sterilization method.

Acknowledgements

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References

[1] S. Ferraris. S. Spriano, Mater. Sci. Eng. C 61 (2016) 965-978.
[2] L. Rimondini, M. Fini *et al*., J. Appl. Biomater. Biom. 3 (2005) 1-10.

IMPROVING ANTIBACTERIAL ACTIVITY OF BONE CEMENT DOPED WITH NANOSILVER

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[ENGINEERING OF BIOMATERIALS 158 (2020) 48]

Introduction

The use of biomaterials, such as acrylic bone cement, is always associated with the opening of the body's layers and contributed to the hospital-acquired infections. Most of these infections are due to a group of multi-drug resistant clinical bacterial strains, mainly *Staphylococcus aureus*, which also produce biofilm [1]. Currently, the gold standard that reduces the adhesion and proliferation of bacterial colonies is doping bone cement with antibiotic/s. However, there is an emerging problem of reduced the antibiotic's effects by biofilm structures and/or increased bacterial resistance [2]. Therefore, more effective solution is sought and particularly noteworthy are nanometals, especially nanosilver. Bone cement containing nanosilver seem to be a good solution, due to better bactericidal properties [2-4]. However, there remains a problem with its proper release from a non-biodegradable and low-porosity cement matrix. In this work, nanosilver-doped bone cement was modified with different biodegradable components to increase nanosilver release and improve its antibacterial properties [5].

Materials and Methods

Acrylic bone cement Cemex (Tecres, Italy) was used as the base material, doped with nanosilver (1.5 wt.%; MkNano, USA) and modified with one of following biodegradable components: cellulose, chitosan, magnesium, polydioxanone and tri-calcium phosphate (5 wt.% Merck, Germany). All bone cement specimens with/without modifications were prepared as described earlier [2-5]. The following tests were performed to evaluate its antibacterial effectiveness: measurement of the turbidity of cultured bacteria broth according to the McFarland standard and measurement of dehydrogenase activity of formed biofilm. *Staphylococcus aureus* strain (ATCC) was used for the tests. Moreover, an analysis of nanosilver release was assessed using the UV-VIS spectrophotometry method.

Results and Discussion

It is possible to incorporate a biodegradable component in acrylic bone cement structure and obtain a time-varying porosity of its matrix. The addition of modifiers did not negatively affect the polymerization temperature and curing time of cements. After one-month exposure to the PBS solution, porosity of modified bone cement significantly improved. The enhanced dissolution of modifiers contributed to the increase of nanosilver release rate indicated in the UV-VIS analysis. The greatest impact on the release of nanosilver had cellulose, tri-calcium phosphate, magnesium and chitosan, after short incubation time (3 days) and cellulose, polydioxanone, magnesium and tri-calcium phosphate, after long time (28 days). The high inhibition of *Staphylococcus aureus* growth in bacterial broth was observed for nanosilver-doped bone cement modified with cellulose and chitosan. While the high inhibition of dehydrogenase activity of *Staphylococcus aureus* biofilm was observed for all modifiers except tri-calcium phosphate.

Conclusions

Our results show that the studied modifiers are suitable for obtaining the partially-biodegradable bone cement and cellulose seems to be the most promising modifier for bone cement doped with nanosilver to improve its antibacterial activity.

Acknowledgments

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References

[1] M. Wekwejt, M. Dziaduszewska *et al.,* Eur. J. Med. Tech. 4 (2018) 19-26.
[2] M. Wekwejt, A. Michno *et al.,* Nanomaterials 9 (2019) 1-18.
[3] M. Wekwejt, N. Moritz *et al.,* Polym. Test. 70 (2018) 234-243.
[4] M. Wekwejt, D. Etmańska *et al.,* J. Biomed. Mater. Res. B. 2020 (2020) 1-10.
[5] M. Wekwejt, M. Michalska-Sionkowska *et al.,* Mater. Sci. Eng. C – in press.

ADSORBED GROWTH FACTORS AS MODULATORS OF CELL BEHAVIOUR ON BIOMATERIAL SURFACES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 49]

Introduction

Currently used or tested synthetic vascular prostheses made of ePTFE, PET or PCL have a limited patency rate for replacement of small-diameter blood vessels. This is caused mainly by poor endothelial cell adhesion and proliferation on the biomaterial surface [1]. It is generally known that a confluent mature endothelial cell layer provides the best prevention of the graft thrombosis, restenosis and failure.

In order to improve the surface properties of synthetic materials for cell colonization, it is suitable to coat the surface with growth factors exhibiting adhesion-enhancing and mitogenic effects [2,3,4].

The purpose of our study was to investigate the effect of basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor A (VEGF-A_{165}) adsorbed to the plastic surface on the behaviour of two cell types which are commonly used in cardiovascular tissue engineering, namely adipose-derived stem cells (ADSCs) and human umbilical vein endothelial cells (HUVECs).

Materials and Methods

FGF-2 and VEGF-A_{165} were expressed in eukaryotic system of methyltrophic yeast *P. pastoris* (strain KM71H) using pPICZαA expression vector.

Cell adhesive properties of the growth factors were monitored with the use of xCELLigence RTCA SP sensing device. Wells in a sensory E-plate were adsorbed with FGF-2 or VEGF (concentration range from 0.01 μM to 10 μM), and the non-specific binding sites for cells were blocked with 0.5% BSA. The initial adhesion of ADSCs and HUVECs was monitored for 4 hours in a pure cultivation medium containing no supplements (DMEM for ADSCs and EBM2 for HUVECs).

To determine the effect of the adsorbed growth factors on the proliferation of HUVECs and ADSCs, wells in 96-well tissue culture plates were coated with the growth factors in concentrations from 0.01 μM to 10 μM. ADSCs were grown in DMEM medium containing 10% of fetal bovine serum (FBS). HUVECs were grown in EGM2 medium containing hydrocortisone, heparin, ascorbic acid and 2% of FBS. The metabolic activity of the cells was evaluated by a resazurin assay on day 1, 3 and 7 after cell seeding. The cells were fluorescently stained with phalloidin-TRITC to visualize the cell morphology after the initial adhesion and during long-term cultivation. The cell nuclei were counterstained with Hoechst 33258.

Results and Discussion

In FGF-2-coated wells, the initial adhesion of ADSCs was significantly elevated. Surprisingly, HUVECs showed no specific interaction with FGF-2-coated wells.

Both studied cell types showed poor adhesion to wells adsorbed with VEGF.

The proliferation and the cell number of ADSCs on immobilized FGF-2 was significantly elevated, reaching the highest values at 10 μM concentration of this factor. A similar effect was also observed in HUVECs (FIG. 1).

On the other hand, ADSCs grown on adsorbed VEGF showed only slight increase in the proliferation and the cell number. HUVECs grown on VEGF-coated wells displayed a higher metabolic activity and a higher cell number. However, the increase in the cell number was not that high when compared with FGF-2-coated wells, where the cell number showed large increase (FIG. 1).

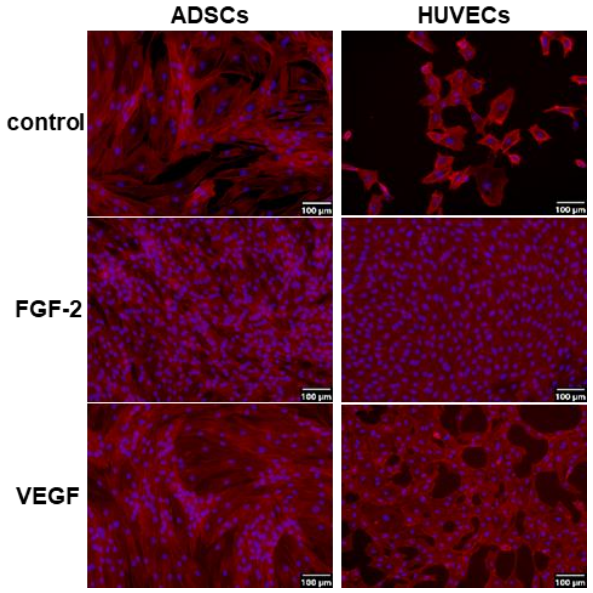

FIG. 1. The morphology of ADSCs and HUVECs 7 days after seeding in wells coated with 10 μM of FGF-2 or VEGF. Scale bar 100 μm.

Conclusions

Our study proved the bioactivity of growth factors adsorbed to plastic surface. Immobilized FGF-2 promoted the proliferation of both cell types and mediated cell type-specific adhesion. Adsorbed VEGF increased the proliferation of HUVECs and displayed poor adhesion-supporting properties. Our results suggest that the coating of the biomaterial surface with FGF-2 and VEGF can direct the adhesion and growth rate of different cell types in different ways.

Acknowledgments

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References

[1] Patel S., Tsang J. *et al.*, Journal of Biomedical Materials Research Part A, 83A (2007) 423–433.
[2] Traub S., Morgner J. *et al.*, Biomaterials. 34 (2013) 5958-5968.
[3] Shin YM, Lee YB *et al.*, Biomacromolecules. 13 (2012) 2020-2028
[4] Kang JM, Han M. *et al.*, Acta Biomater. 8 (2012) 1759-67.

BIOACTIVE CERAMIC COATING FORMED ON Ti BONE WEDGE

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[ENGINEERING OF BIOMATERIALS 158 (2020) 50]

Introduction

Bone tissue implant material should be characterised by good biocompatibility ensuring the osseointegration of implant and bone and shows appropriate mechanical properties [1]. Bone wedge implant usually are made by Ti-6Al-4V alloy. There is a wide range of surface modification methods which aim is to enhance the osseointegration between implantable material and bone tissue. One of the promising surface treatment is the plasma electrolytic oxidation (PEO) process [2]. The foundation of PEO treatment is an application of voltage higher than oxide layer breakdown voltage during the anodization and a coating with characteristic morphology is formed. The formed oxide layer can be additionally enriched with elements or compounds from the anodizing bath increasing the bioactivity and biocompatibility of the implant. Coating formation using the sol-gel technique allows the design of a surface with desirable chemical and phase compositions [3]. The aim of this work was formation ceramic coatings on the bone wedge Ti implant for animal bone.

Materials and Methods

The Ti bone wedge (IWET, Poland) was anodized in 0.1 M $Ca(H_2PO_2)_2$ solution (Alfa Aesar, Germany) with $Ca_3(PO_4)_2$ (Avantor, Poland) particles at 350 V. Next, an additional sol-gel coating was formed on the previously anodized sample from solutions composed of hydroxyapatite precursors. The bone wedge was heat treated for 30 min in a furnace at 660°C with a controlled ramp rate (10°C/min) in the air condition. The morphology of the modified sample was examined by scanning electron microscopy (SEM) (Hitachi; TM-3000; BSE mode). A cross-section of the implant was also analyzed, and the surface roughness and wettability, as well [4]. Evaluation of titanium alloy sample cytocompatibility was carried out using human osteoblast-like MG-63 cells seeded at an initial density of 8,000 cells per sample (representative samples of medical Ti-6Al-4V alloy with the ceramic coatings). Cell metabolic activity, attachment and distribution of the adhered cells were evaluated as described previously [4].

Results and Discussion

The titanium bone wedge implant was anodized in a $Ca_3(PO_4)_2$ suspension, and then the additional layer was formed by the sol-gel technique to obtain a mixture of the calcium phosphate compounds (FIG. 1A). The oxide layer was porous, and additional ceramic particles were formed after sol-gel treatment (FIG. 1B). After the sol-gel process the Ca/P content in the total ceramic layer increased up to 0.89 (FIG. 1C). Cross-section analysis of the coatings also confirmed that the thickness of the ceramic layer increased around 5 μm after additional posttreatment process. Formation the additional ceramic particles on the top of the implants surface caused that the surface roughness increased from 0.91 μm ±0.21 to 1.07 μm ±0.11. The average surface roughness was determined based on 2D and 3D profiles of the SEM images (FIG. 1D). Wettability of the surface was also changed to become more hydrophilic, which is favourable for bone cell adhesion.

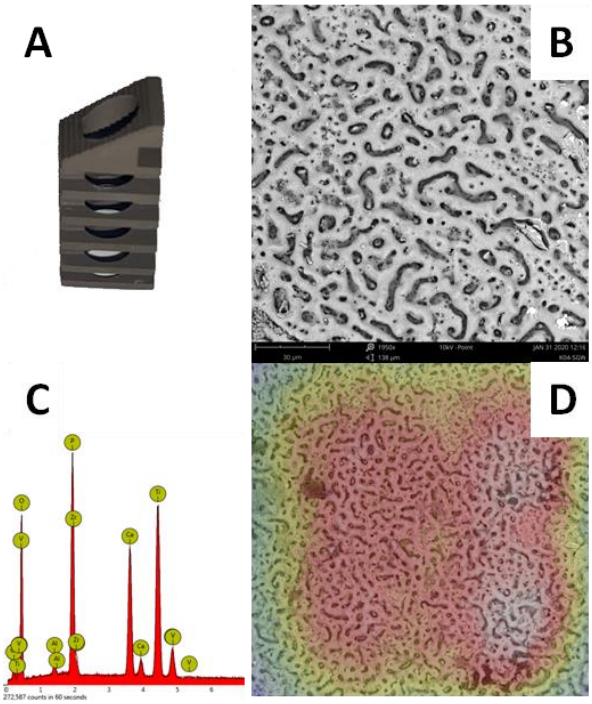

FIG. 1. Image of a bone wedge with ceramic coating (A), surface morphology of the modified implant surface (B), EDX analysis of the coating (B) and their surface roughness profile analyzed based on the SEM images (D).

Surface modification such as formation of the hybrid ceramic layer influenced the differences in the number of osteoblast-like MG-63 cells compared with the unmodified titanium sample. The cells were well adhered and their number increased with time of their culture.

Conclusions

The results showed that a hybrid ceramic layer containing amount of calcium and phosphate-related compounds can be formed on the bone wedge implant. Application of the plasma electrolytic oxidation process and sol-gel technique is favourable for surface treatment of the animal implant for bone tissue. The hybrid, ceramic coating was cytocompatible with osteoblast-like MG-63 cells.

Acknowledgments

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References

[1] M. Geetha *et al.,* Prog. Mater. Sci. 54 (2009) 397-425.
[2] K. Rokosz *et al.,* Materials 13 (2020) 2468 - 2490.
[3] L. Le Guéhennec *et al*., Dent Mater. 23 (2007) 844-54.
[4] A. Kazek-Kęsik *et. al*., Mater. Sci. Eng. C Mater. Biol. Appl. 94 (2019) 998-1008.

HYDROGELS CROSSLINKED WITH NATURAL COMPOUNDS FOR BIOMEDICAL APPLICATIONS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 51]

Introduction

In modern medicine there is a plenty of space for application of hydrogels, including drug carriers and tissue engineering scaffolds. There is also a wide choice of polymeric materials that can form those specific networks with high water content. Recently, special attention has been given to natural polymers, e.g. starch, cellulose, hyaluronic acid or chitosan. The latter, despite being a natural polysaccharide, is usually crosslinked using toxic compounds, like glutaraldehyde what significantly limits biomedical potential of chitosan-based hydrogels. The solution would be to find an effective crosslinker among non-toxic, natural compounds.
The aim of this study was to examine properties of the chitosan-based hydrogels crosslinked with vanillin. Blends of chitosan, poly(vinyl alcohol) and gelatin were tested along with composites modified with graphene oxide and hydroxyapatite.

Materials and Methods

Blends of chitosan (CS: Sigma-Aldrich, low molecular weight), gelatin (GEL: Avantor Performance Materials Poland S.A.) and poly(vinyl alcohol) (PVA: Avantor Performance Materials Poland S.A.) were prepared with various ratios (1:1:1, 3:1:1, 1:3:1, 1:1:3, respectively) for reference and then blends with two different weight ratios of CS:vanillin (0.5:0.3 marked as van1 and 0.5:0.4 marked as van2) were obtained as well. For composite samples, graphene oxide (GO, 1 wt%: ITME, Poland) and hydroxyapatite (HAp, 10 wt%: Chema Elektromet) were dispersed in the polymer solution using sonication. Samples were subsequently frozen in 24-well plates and freeze-dried (FIG. 1).

FIG. 1. Digital microscope image of freeze-dried 1CS_1GEL_1PVA_GO_HAp sample.

The influence of natural crosslinkers on crosslinking process was studied. Rheological properties of the solutions used to obtain the materials were examined. Mechanical properties of the blends were evaluated in a static compression test, thermal properties were studied by DSC (differential scanning calorimetry) technique. Fourier transform infrared (FTIR) spectroscopy was used to assess the structure. Surface of the samples incubated in simulated body fluid (SBF) was analyzed using a scanning electron microscope (SEM).

Results and Discussion

The rheological studies have clearly shown that the addition of vanillin increases the viscosity of the chitosan solution (FIG. 2). Moreover, FTIR results confirmed that the aldehyde group of vanillin interacts with the amino group of CS via Schiff base reaction, hence creating three dimensional hydrogel network. FTIR revealed also presence of some interactions between CS and GO. Generally, samples crosslinked with higher amount of vanillin (van2) and those with GO were more stable than their respective reference samples. It confirms not only that vanillin can act as crosslinker but suggests also that the GO plates can play similar role.

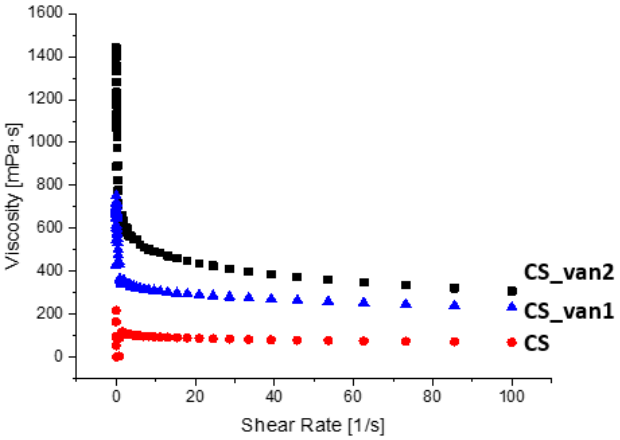

FIG. 2. Viscosity flow curve of the CS-based solution uncrosslinked and crosslinked with different amounts of vanillin.

Preliminary bioactivity assessment (SBF, 37°C) confirmed that calcium phosphates were formed on the surface of all the samples although their morphology differed (FIG. 3).

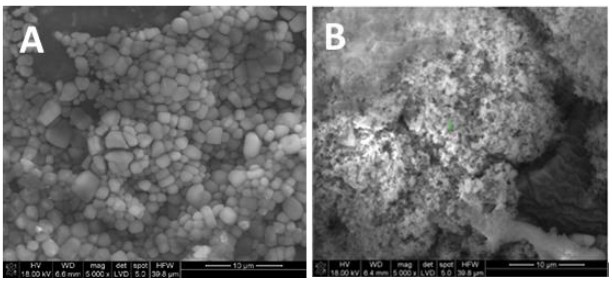

FIG. 3. SEM images of (A) 1CS_3GEL_1PVA_van2 and (B) 3CS_1GEL_1PVA_van2 ater 2 weeks of incubation in SBF.

In the end, two hydrogel systems based on 1CS:1GEL:1PVA and 3CS:1GEL:1PVA blends modified with GO and HAp. and crosslinked with vanillin (van2) were singled out for further studies as the most promising materials.

Conclusions

Vanillin can be used as a chitosan crosslinker due to the presence of aldehyde groups. Moreover, this organic compound is of natural origin and was proven to have bioactive properties. Chitosan-vanillin systems can be exploited in various biomedical applications.

Acknowledgments

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THE INFLUENCE OF MICROSTRUCTURE OF FIBROUS PLA/PVA MEMBRANES ON PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 52]

Introduction

Nano- and sub-micron scale of fibrous materials produced by electrospinning method draw the attention of many researchers. Versatility, affordability and fiber architecture comparable to natural extracellular matrix is the greatest asset of this technique. This fiber support cell adhesion, proliferation and differentiation of every tissue. However, mimicking the three-dimensional network of the native ECM is still challenging [1]. Dense packing of the fibers and small pores diameter limit cell infiltration to inner side of the scaffold. Fortunately, highly versatile electrospinning method led to the formation fibrous scaffold with enhanced pore size. One of the approach to increasing pore size is use of sacrificial fibers in two-syringe system electrospinning [2]. Scaffold composed of soluble as well as insoluble polymer fibers and in the sequel selective removal of the soluble elements enhance the pore size [3]. This two-syringe system could deposit fibers layer by layer and due to the time lag tailored the gap between insoluble fibers. Alternatively, bi-modal scaffold could be also fabricated by concurrently electrospinning. Typically, the sacrificial element is removed but also it might well form a part of drug carrier. Based on the fiber diameters, the size of the gap could be successfully controlled. As a result of sequential or concurrent electrospinning, electrified polylactide and polyvinyl alcohol fibers can be obtained. The fibers diameter was tailored by several process parameters e.g. molar mass or distance between the tip of the needle and collector [4].

Materials and Methods

PLA (3251D and 3001D, Nature Works) was dissolved in dichloromethane (DCM, Avantor) and dimethylformamide (DMF, Avantor). Distilled water and ethanol (EtOH, 98%) were the solvents for PVA (M.W. 500-5000 and 115000 Alfa Aesar). Morphology was characterized used the scanning electron microscope (NOVA NANO SEM 200). Physicochemical properties of the surface: wettability, surface energy was tested using a goniometer (DSA 25 Kruss). The presence of two different polymer fibers was confirmed by Fourier transformation infrared spectra (FTIR, FT 3000 Excalibur). The porosity of the manufactured membranes was determined by mercury perimetry (PoreMaster 60, Quantachrome). In vitro cell response was performed by seeding fibroblast on the fibrous membrane.

Results and Discussion

Expected results differences in fibers diameter and the porosity of the non-woven mats depending on different work distance and molecular mass which affect on inner pore size. Altering distance between tip of needle and collector, fiber diameters can be varied by 400nm and 40nm for PLA and PVA, respectively (FIG. 1). That parameters correlate with porosity. Depending on polymer and type of bi-modal electrospinning the value of the water contact angle is in the range from 70 to 130° (TABLE 1, FIG. 1). FTIR spectra confirming the presence of both polymer fibers in the membrane (FIG. 2). The absorption bands from -OH and C-H groups confirms the presence of PVA fibers in the bi-modal membrane. Characteristic C=O, C-O and O-C-O groups for PLA are also present.

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

Type of two-syringe electrospinning		Contact angle [°]
Sequential	1	131,11°± 6,77
	2	73,01°±5,24

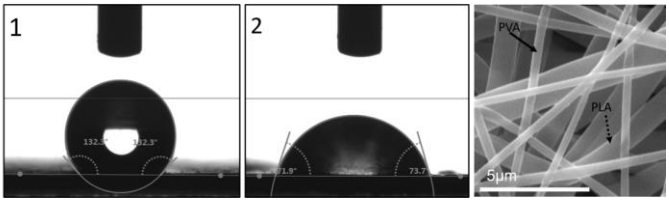

FIG. 1 The picture of water droplet sits on bi-modal scaffold consist of: 1 – PLA/PVA/PLA and 2 – PVA/PLA/PVA. SEM microphotography of PVA and PLA fibers obtained by sequential electrospinning (PVA/PLA/PVA).

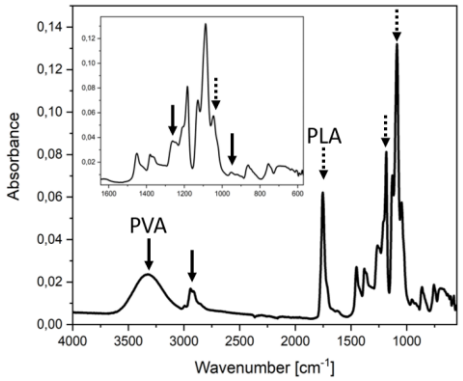

FIG. 2. FTIR spectra of PVA/PLA/PVA bi-modal scaffold.

Conclusions

Electrospun membrane with enhanced porosity can be tailored to provide sufficient scaffold for dermal tissue engineering. Two approaches of sequential and concurrent bi-modal membrane have different interaction with dermal fibroblasts and it have different physical properties.

Acknowledgments

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References

[1] E. Stodolak-Zych *et al.*, J. Mol. Struct., 1211 (2020) 128061.
[2] Z. Tan *et al*., Mater. Sci. Eng. C, 67 (2016) 369-377.
[3] J. Ameer *et al*., J. Funct. Biomater., 10 (2019) 30.
[4] J. Rnjak-Kovacina *et al*., Biomaterials, 32 (2011) 6729-6736.

FIBROUS CARBON SCAFFOLD MODIFIED BY POLYSACCHARIDES WITH POTENTIAL BIOLOGICAL ACTIVITY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 53]

Introduction

In medicine carbon fibers are mainly known as a component of complex, multi-phase composites. At the same time, carbon fibers themselves in the form of nonwovens or mats can be attractive scaffolds facilitating cell adhesion, proliferation and differentiation. Thus, fibrous materials of different fibres distribution i.e.: unidirectional (1D), bidirectional (2D) or multidirectional (MD) may constitute scaffolds for the cells of many tissues [1]. Due to high biocompatibility of amorphous carbon fibres, these substrates are an interesting biomaterial, which can be used independently or additionally modified to enhance and direct the biocompatibility effect towards a specific cell group [2-3].
The potential of carbon fibres to regenerate bone tissue can be regulated by their modification with biopolymers. Chitosan (CS), alginate (CA) or gelatin (G) are known for their ability to support repair processes in damaged cartilage. It seems that the combination of carbon fibres and polysaccharides will facilitate the stimulation of cartilaginous growth, especially in areas where there is a contact between two tissues: cartilage and bone, e.g. in cartilaginous dysplasia.
The aim of this study was to obtain and characterize low-module carbon nonwovens, which were references for fibrous constructions being a combination of carbon nonwoven fabric and one of the selected polysaccharides: chitosan (CF/CS) or alginate (CF/CA).

Materials and Methods

Controlled thermal conversion of polymeric nonwoven fabric obtained from polyacrylonitrile by the wet spinning method (Lukasiewicz Research Network - The Textile Research Institute) to carbon fibers in two-stage process: oxidation (250°C/air) and low-temperature carbonization (970°C/nitrogen) was conducted. Fiber morphology was observed using scanning electron microscope (Nova NanoSEM, FEI). A change in the wettability of materials related to the method of thermal treatment was shown; the longer the oxidation stage, the higher the hydrophilicity of materials (up to 65°, Kruss goniometer 25E) and the more numerous oxygen functional groups (carboxylic, carbonylic groups, FTIR-ATR, Bruker BioRAD).

Results and Discussion

During thermal conversion process material were shrinkage (about 12% of total shrinkage of nonwoven fabric, about 6% shrinkage of a single fiber) was recorded. A change in the wettability of materials related to the method of thermal treatment was shown; the longer the oxidation stage (with low temperature ie. 180°C), the higher the hydrophilicity of materials (up to 65°) and the more numerous oxygen functional groups (carboxylic, carbonylic groups). Shorter oxidation stage at higher temperature (260°C/30min) reduces the hydrophilicity of the material but allows to obtain a less brittle non-woven fabric, which makes further processing easier (carbonization, 970°C).
Carbon nonwovens have a wettability of 70-80°, which makes them easy to contact with biopolymer solutions. Soaking the non-woven fabric in polymer solution and its lyophilization leads to maintaining the fibrous form of the structure and allows easier contact of the materials with the culture medium. The biopolymer layer present on the fibres does not exceed a few micrometers (1-3 μm) and depends on the concentration of the biopolymer with which the nonwoven is soaked.
The wettability of the carbon fibric - biopolymer system changes in relation to the unmodified nonwoven; the substrate becomes more hydrophobic (about 90°). The biopolymer layer limits material crumbling and facilitates its portability during manipulations connected with preparation of material for biological tests in in vitro condition (sterilization, microscopic observations).

Conclusions

The complex two-component systems: carbon fiber - biopolymer (alginate, chitosan) allow to obtain structures with increased stiffness of CF/CA and CF/CS, which facilitates their manipulation during biological tests and clinical applications. Further work on these materials is necessary to determine their medical suitability.

Acknowledgments

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References

[1] S.K. Nataraja *et al.,* Prog. Polym. Sci., 37 (2012) 487-513.
[2] E. Stodolak-Zych, *et al.,* J. Mol. Struct., 1126 (2016) 94–102.
[3] S. Singh, *et al.,* Mater. Sci. Eng. C, 38 (2014) 46–54.

ELECTROPHORETIC DEPOSITION AND CHARACTERISATION OF SODIUM ALGINATE COATINGS ON COMMERCIALLY PURE TITANIUM

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[ENGINEERING OF BIOMATERIALS 158 (2020) 54]

Introduction

Titanium and titanium alloys are a very important group of metallic biomaterials. They have low density, relatively low elasticity modulus, good electrochemical corrosion resistance and good biocompatibility. These materials are generally used in orthopaedic surgery and dentistry. On the other hand, their application as bone implants is restricted by poor osseointegration [1]. To improve the direct bone-to-implant contact, bioactive and biodegradable coatings are often applied. In this work, sodium alginate coatings were fabricated on titanium substrates by electrophoretic deposition (EPD). EPD is a surface engineering method used for the deposition of polymeric, ceramic and composite coatings, especially for biomedical applications [2]. Sodium alginate is one of the most important biopolymers. Due to its biodegradability, biocompatibility and non-toxicity, it is widely used as a matrix of composite coatings [3]. The aim of the present work was to elaborate the conditions of EPD of pure sodium alginate (SA) coatings on commercially pure titanium and to characterise the coating microstructure, surface topography and selected properties.

Materials and Methods

A commercially pure titanium CP-Ti Grade 1 was used as a substrate material for coating deposition. Two different ways of substrate preparation were used before deposition, (i) in as–received condition and (ii) after chemical treatment by washing in acetone, soaking in a 0.06 M solution of $Na_3PO_4 \cdot 12H_2O$ at 80 ºC, washing in hot water and soaking in a solution of 5 ml HF 40% + 35 ml HNO_3 70% in 60 ml H_2O for 5 minutes. Synthetic SA powder was used as a coating component. The suspension used for EPD consisted of a different concentration of SA of 2 g/l, 4 g/l or 8 g/l in a different volume ratio of distilled water to EtOH of 20/80, 40/60 and 60/40. The counter electrode was made of austenitic stainless steel (AISI 316L). The EPD was performed at the constant voltage of 3, 5, 7, 10 and 12 V for the deposition time of 300 s. The zeta potential (ZP) was measured for the real suspensions in the pH range of 3-12. The microstructure of the coatings was investigated by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The surface topography of the coatings was analysed by optical profilometry and by atomic force microscopy (AFM). The contact angle and surface free energy (SFE) were investigated. The adhesion of the coatings to the titanium substrates was determined by a cross cut adhesion test in accordance with ASTM D3359-B.

Results and Discussion

The homogeneity of coatings obtained by EPD can be controlled by the chemical composition of the suspension and process parameters. It was found that, in the case of a dispersion phase consisting of the distilled water to EtOH volume ratio of 20/80 or 40/60, the precipitation of SA was observed for all the investigated concentrations of SA. The zeta potential of the suspensions consisting of distilled water and EtOH in the volume ratio of 60/40 containing a different concentration of SA equalled 2 g/l, 4 g/l and 8 g/l and was negative in all the investigated pH range of 3-12. Thus, the SA particles were negatively charged and moved towards the anode under the electric field. The highest values of ZP for different concentrations of SA (2, 4 and 8 g/l) were -56.1 mV (for pH=10), -66.0 mV (for pH=9) and -90.6 mV (for pH=7), respectively. Macroscopically homogeneous coatings were obtained from the suspension containing 4 g/l and the volume ratio of distilled water to EtOH of 60/40 at the voltage of 10 V during 300 s. The coatings deposited from the suspension containing 2 g/l of SA were thin and not uniform. The coatings deposited from the suspension containing 8 g/l contained numerous gas bubbles. After EPD at a voltage lower than 10 V, the coating was not continuous and only partially coated the substrate, but if the voltage was higher than 10 V the substrate oxidized. The thickness of the coatings deposited from the suspension containing 4 g/l of SA was in the range of 840–980 nm. Electron microscopy investigation revealed that the coatings were dense and homogeneous. It was found that they were characterized by average surface development. Selected surface topography parameters of the coatings, e.g. R_a (the average roughness), R_q (the root mean square roughness) or R_{max} (maximum vertical distance between the highest and lowest point) equalled 197.4 ± 60.1 nm, 254.0 ± 80.2 nm and 1651.6 ± 521.0 nm, respectively. Tape tests showed that the coatings revealed slightly better adhesion to the chemically treated titanium substrates than to the as-received ones. The coatings exhibited a hydrophilic character. The contact angle for distilled water and diiodomethane equalled 47.7 ± 5.8 and 46.5 ± 3.6, respectively, while SFE equalled 57.3 ± 5.6.

Conclusions

Sodium alginate coatings were successfully deposited on titanium substrates by EPD. Uniform coatings were obtained from the suspension containing 4 g/l of SA in a volume ratio of distilled water to EtOH of 60/40 at a potential difference of 10 V during 300 s. The obtained coatings were homogeneous and exhibited average surface development. The coatings exhibited slightly better adhesion to the chemically treated substrates in comparison to the as-received substrates. The coatings exhibited a hydrophilic character. Further optimisation of the substrate preparation and characterisation of the coatings are in progress.

Acknowledgments

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References

[1] L. Salou, A. Hoornaert, G. Louarn, P. Layrolle, Acta Biomater., 11 (2015) 494 - 502
[2] A. R. Boccaccini, S. Keim, R.Ma, Y. Li, I. Zhitomirsky, J. R. Soc. Interface, Suppl 5 (2010) 581 - 613
[3] Q. Chen, L. Cordero – Arias, J. A. Roether, S. Cabanas – Polo, S. Virtanen, A. R. Boccaccini, Surf. Coat. Technol. 233 (2013) 49 - 56

WHEY PROTEIN ISOLATE HYDROGEL-BASED BIOMATERIALS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 55]

Introduction

Whey Protein Isolate (WPI) is a byproduct from the dairy industry whose main component is beta-lactoglobulin (β-LG). When added to cell culture medium, WPI has stimulated the proliferation and osteogenic differentiation of human-derived adipose stem cells and also osteoblastic cell lines [1,2].

Hence, we hypothesized that biomaterials fabricated from WPI would support cell growth.

Upon heating of a WPI solution, hydrogels form thanks to formation of crosslinks between β-LG molecules. Importantly, these hydrogels can be sterilized by autoclaving, an important practical advantage.

A range of cells have been successfully cultivated on WPI hydrogels in the concentration range 20-50% (w/v). Furthermore, it is possible to improve the biological performance of WPI hydrogels by incorporation of a mineral phase, e.g. by addition of particles of aragonite [3] or alpha-tricalcium phosphate (α-TCP) [4]. It is also possible to induce mineralization of WPI hydrogels with calcium and magnesium carbonates using urease [5] or with phosphates by using alkaline phosphatase (ALP) immobilized on particles to prevent heat denaturation. By adding other particles, such as carbon nanotubes (CNT), it is possible to impart antibacterial activity. Furthermore, it is possible to solubilize hydrophobic molecules with biological activity (e.g. polyphenols such as Tannic Acids (TAs), phenolic compounds like phloroglucinol (PG), the phenolic subunit of phlorotannins, which are polyphenols found in brown seaweeds) within the WPI hydrogel network to endow hydrogels with antimicrobial activity.

This work will summarize several of the strategies mentioned above.

Acknowledgments

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References

[1] Douglas TEL et al, J Dairy Sci. 2018;101(1):28-36.
[2] Carson M et al, Cells Tissues Organs. 2018;206(1-2):106-118.
[3] Gupta D et al, Materials and Design 2020, 188, 108408
[4] Dziadek M et al, J Biomed Mater Res A. 2019;107(11):2479-2491.
[5] Norris K et al, Mar Drugs. 2020;18(6):294.

THE INFLUENCE OF COCAMIDOPROPYL BETAINE ON THE MECHANICAL AND THERMAL PROPERTIES OF POLYMER FILMS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 56]

Introduction

Polymer blending is one of the effective methods for providing new desirable polymeric materials for a variety of applications [1]. Several studies have been reported to blend natural polymers such as chitosan with synthetic ones for example poly(vinyl alcohol) (PVA) [2-4]. Chitosan and PVA create intermolecular hydrogen linkages among their polymer chains [5].

PVA is a polyhydroxy water-soluble polymer, non-toxic, biocompatible polymer with film-forming capacity and high tensile strength and flexibility [6,7]. PVA was used in many applications such as membrane preparations, food packaging, drug delivery systems, etc. [8,9].

Chitosan (CTS) is a natural polysaccharide, a derivative of chitin which is obtained from the exoskeleton of invertebrates [10]. Based on the characteristics of CTS, including antimicrobial activity, biodegradability, and UV-protective ability, it can be used for the preparation of films for various purposes [11].

Surfactants are widely applied to organic chemicals as they are one of the main ingredients in personal hygiene products and detergents [12]. Cocamidopropyl betaine (CAPB), belonging to the amidopropyl betaine group, is an amphoteric surfactant which has two ionic centers of different charge in one molecule (FIG. 1). CAPB shows a cleaning effect and acts as a foaming agent [13].

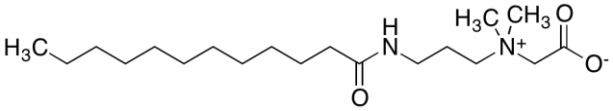

FIG. 1. Structure of cocamidopropyl betaine.

The aim of this study was to develop materials based on biodegradable polymers (PVA and chitosan) with the addition of the surfactant (cocoamidopropyl betaine) to provide the film washing properties.

Materials and Methods

Chitosan (CTS) and poly(vinyl alcohol) (PVA) were purchased from Sigma-Aldrich (Poznan, Poland). Cocamidopropyl Betaine (CAPB) was acquired from CHEMCO (Sobowidz, Poland). The molecular weight of PVA was 31,000-50,000 g/mol and the hydrolysis degree was about 98-99%. The deacetylation degree of chitosan was ≥75% and the average molecular weight was equal to 444,000 g/mol.

The films were fabricated from chitosan, poly(vinyl alcohol), and surfactant (cocamidopropyl betaine) using a casting solutions technique. Chitosan solution (2% w/w) and PVA solution (2% w/w) were mixed. The addition of the surfactant was 2%. The PVA/CTS films were prepared at various weight ratios to evaluate the optimum composition. The samples have been dried at room temperature for 7 days.

The properties of the polymer matrices were determined by mechanical tests and thermogravimetric analysis. The mechanical tests were conducted at room temperature using a mechanical testing machine equipped with tensile grips. Young's modulus and elongation at break were calculated. Thermal stability was performed by the thermogravimetric instruments in nitrogen with a heating rate of 10°C/min up to 600 °C.

Results and Discussion

The obtained results showed that the PVA/CTS films exhibited much higher values of Young's modulus compared to the matrices with the surfactant. The neat polymer blends were very stiff, while the PVA/CTS+CAPB films were more flexible. Moreover, the values of elastic moduli were also dependent on the composition of the prepared materials. The higher amount of chitosan increased the stiffness of the obtained matrices. It was noted that the PVA/CTS films had slightly lower elongation at break values than the films with the addition of cocamidopropyl betaine. It indicates that the prepared materials were brittle. The weight ratios of the polymers in the samples had an impact on the elongation at break values. The lower amount of PVA in the blends increased the fragility of the matrices. Thermal behavior of the studied blends depended on their composition. Higher content of PVA in the blends resulted in higher temperature at maximal rate decomposition of the samples. For the blends with 50 and 75% of PVA and the surfactant, the temperature at maximal rate process enhanced but for the blend with 25% of PVA and the surfactant, the temperature slightly decreased. Moreover, the presence of CAPB made the weight loss in the first stage more efficient.

Conclusions

The prepared matrices based on biodegradable polymers with the addition of surfactant can be the starting point of producing novel materials to replace currently used wet wipes made of non-degradable polymers.

Acknowledgments

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References

[1] K. Makhijania, R. Kumara, S.K. Sharma, Crit. Rev. Environ. Sci. Technol. 45 (2015) 1801-1825.
[2] M. Koosha, S. Hamedi, Prog. Org. Coat. 127 (2019) 338-347.
[3] S-D. Yoon, Y-M. Kim et al. J. Photochem. Photobiol. B. 176 (2017) 145-149.
[4] Y. Liu, S. Wang, W. Lan, Int. J. Biol. Macromol. 107 (2018) 848-854.
[5] W. Lan, S. Wang et a. Int. J. Biol. Macromol. 145 (2020) 722-732.
[6] A.A.Menazea, A.M.Ismail et. al. J. Mater. Res. Technol. 9 (2020) 9598-9606.
[7] A. Jayakumar, K.V. Heera et al. Int. J. Biol. Macromol. 136 (2019) 395-403.
[8] D. Huang, Z-D. Hu et al. Polym. Degrad. Stabil. 163 (2019) 195-205.
[9] A. Abrahama, P.A. Solomanb, V.O. Rejini, Proc. Technol. 24 (2016) 741-748.
[10] X. Ma, C. Qiao et al. Int. J. Biol. Macromol. 135 (2019) 240-245.
[11] N.E. Kochkina, N.D. Lukin, Int. J. Biol. Macromol 157 (2020) 377-384.
[12] A. Gholami, M. Golestanehb, Z. Andaliba, Spectrochim. Acta A 192 (2018) 122-127.
[13] M. Merkova, M. Zalesak et al. Int. Biodeterior. Biodegradation 127 (2018) 236-240.

TECHNOLOGY SELECTION OF SURFACE MODIFICATION FOR CARDIAC IMPLANTS USED IN MCS THERAPY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 57]

Introduction

Currently the Mechanical Circulatory Support (MCS) including the Ventricular Assist Devices (VAD) [1] is considered to be a reliable and effective treatment for patients with advanced heart failure (HF). Moreover very often it is the only possible option for patients waiting for heart transplant. After over 50 years of work on MCS devices many new constructions were introduced to the clinic. Recently the extracorporeal pulsatile VAD's are replaced by new generations of fully implantable continuous flow (CF) pumps with non-contact levitating rotor [2]. The solutions currently used in the clinic use the rotor levitation technology, allowing for non-contact work. Clinical experience has provided information that despite many undeniable benefits, new constructions still require improvement to minimize the risk of complications during heart assistance [3,4]. One of complications are the pump thrombosis and inflow obstruction, caused by the ingrowth of tissue into the lumen of inflow cannula [5-7]. The developers of the actual constructions have proven that surface modification allows to control the tissue ingrowth of the external surfaces of the inflow cannula [8–10]. In comparison smooth surface of the cannula results in tissue overgrowth into the lumen flow and may be a source of emboli.

Materials and Methods

The paper presents additive and subtractive technologies of surface modification of titanium alloy for the external surface of the VADs inflow cannula. The proposed technologies included abrasive blasting [AB], laser ablation [LA], atmospheric plasma spraying [APS], powder sintering [PS]. Samples were prepared from titanium alloy Ti6Al7Nb in form of cylinders Ø14mm x H 3mm. The base material was verified for compliance with the standard including the microstructure study, the chemical composition analysis and the study of mechanical properties. The samples were subjected to tumbling before performing modifications. The roughness was measured with the use of contact profilometry. The base material was characterised by Ra=1,5µm and Rz=12,5µm. The aim of the study was to obtain the surface characterized by high roughness with the potential to implant cells, enabling the formation of scar tissue. During the process of all technologies many parameters were subjected to change including: crystal shape and size, medium pressure, angle of the nozzle, power and diameter of the laser beam, size and shape of powder grains. The obtained surfaces were characterized by wettability, contact profilometry, digital microscope [DM] and scanning electron microscope [SEM].

Results and Discussion

The results have shown that surface after PS is characterized by highest roughness of about ≈ Ra=35µm, high porosity ≈64% and wettability ≈100°. The cross section images obtained with the use of DM revealed its complex 3D morphology. Whereas SEM mages highlighted the presence of empty micro spaces that may stimulate cell growth (FIG. 1).

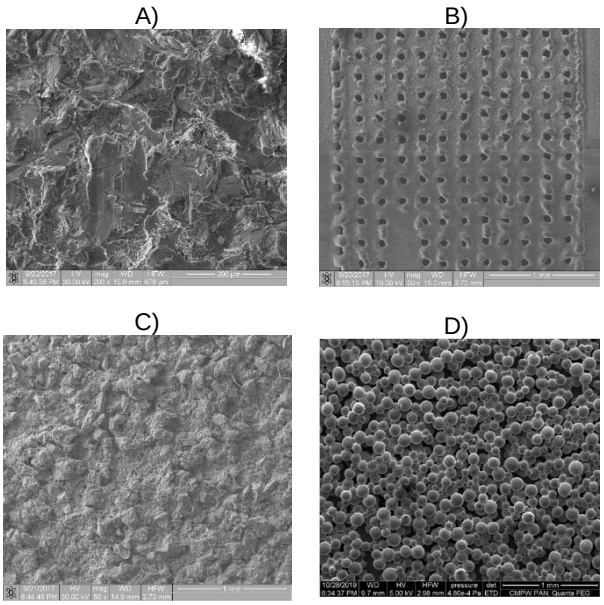

FIG. 1. Sample SEM images for different technologies: A) abrasive blasting [AB], B) laser ablation [LA], C) atmospheric plasma spraying [APS], D) powder sintering [PS]

Conclusions

The surface developed with PS is the most promising for stimulating cardiomyocytes to grow due to its complex 3D morphology, high degree of roughness and porosity. However it is still necessary to perform in vitro tests in terms of cytotoxicity and proliferation, which will be conducted as the next step of the research with the use of fibroblasts and endothelial cells.

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References

[1] J. H. Kim, *Cardiology Clinics*, vol. 36, no. 4. W.B. Saunders, pp. 443–449, 01-Nov-2018.
[2] M. Gawlikowski *et al.*, *Photonics Lett. Pol.*, vol. 12, no. 2, pp. 46–48, 2020.
[3] R. Antończyk *et al.*, *Kardiochirurgia i Torakochirurgia Pol.*, vol. 14, no. 1, pp. 76–78, 2017.
[4] A. B. Nguyen et al., *Curr. Heart Fail. Rep.*, vol. 13, no. 6, pp. 302–309, Dec. 2016.
[5] C. H. Glass et al., *Cardiovasc. Pathol.*, vol. 38, pp. 14–20, Jan. 2019.
[6] M. Gawlikowski *et al.*, Proceedings of SPIE, Volume 10455, Article number 104550L, p. 22, 2017.
[7] S. S. Najjar *et al.*, *J. Hear. Lung Transplant.*, vol. 33, no. 1, pp. 23–34, Jan. 2014.
[8] E. A. Rose *et al.*, *Circulation*, 1994, vol. 90, no. 5 II.
[9] P. Kurtyka *et al.*, Eng.of Biomaterials, Vol. 22, no.151, 2019.
[10] C. M. Zapanta *et al.*, *ASAIO J.*, vol. 52, no. 1, pp. 17–23, Jan. 2006.

INFLUENCE OF SURFACE MODIFICATION OF 316L MEDICAL STEEL ON MICROBIAL ADHESION

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[ENGINEERING OF BIOMATERIALS 158 (2020) 58]

Introduction

316L steel is a material that is often used in the production of medical instruments. When examining the microbiological properties of such materials, efforts are made to reduce the adhesion of bacteria to their surface in order to avoid negative reactions in contact with the human body. One way to achieve the reduction of microbial adhesion to the surface is to reduce the contact area between pathogens and material using various methods of surface topography modification. The investigations presented in the article are a part of this trend of research on the impact of surface modification of 316L steel on microbial adhesion [1].

Materials and Methods

The test samples were divided into three groups depending on the method of surface preparation. The first one was 316L steel (reference test). The second group was subjected to the electro-etching process in an aqueous solution containing 100 g/l H_2SO_4, more than 15 g/l Fe3+, 25 g/l HF and about 1 g/l of additives (emulsifiers, wetting agents, corrosion inhibitors) [2]. The third group was mechanically processed on rotary grinders. All samples before etching were cleaned and washed in 98% acetone in an ultrasonic cleaner. The etching of 316L steel surface was performed with a mixture of nitric and hydrofluoric acids (10% HNO_3 and HF 5%). The etching temperature was 50°C with continuous mixing for 5, 10, 15 minutes consecutively.
The bacteria used in the study came from the American Type Cultures Collection: *Staphylococcus aureus* ATCC 25922, *Escherichia* coli *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. The contact angle was measured using a goniometer with DROPimage Advanced software. The antimicrobial activity of the coatings was assessed by a direct method based on the criteria in ASTM E2922. The susceptibility of the coating surface to microbial adhesion was assessed in accordance with the procedures contained in the ISO 22196: 2011 standard with modifications related to the assessment of microbial viability.

Results and Discussion

The assessment of the contact angle of the tested samples showed their hydrophilic character. The contact angles range from about 50 degrees (49.1) to over 80 degrees (80.8). Samples whose surface was not pre-treated (reference) and mechanically ground show the same tendency. The contact angle increases with increasing chemical etching time. The trend is reversed for samples subjected to the electro-polishing process - the contact angle decreases with increasing etching time.
The assessment of bacterial adhesion to the surface of 316L steel (FIG. 1, TABLE. 1) showed the highest adhesion to the surface of unmodified (reference) samples, and to samples after a treatment time of 5 minutes, for both surface preparation methods, ie. electro -polishing and mechanical grinding. Of the tested bacteria, Escherichia coli was found to be the most numerous on the tested samples.

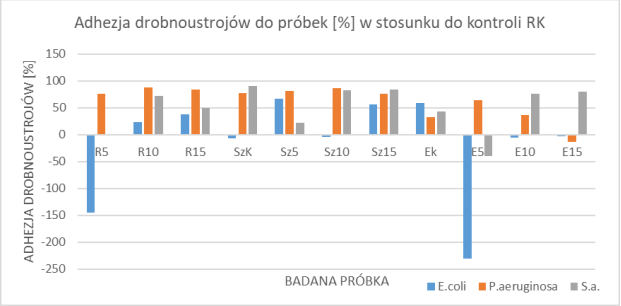
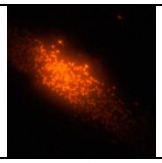
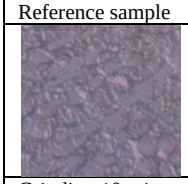
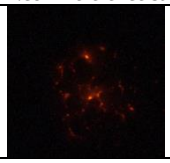
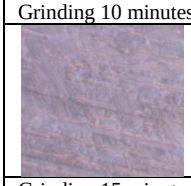
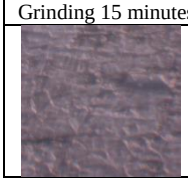


FIG. 1. Microbial adhesion to samples.

TABLE 1. Microbial adhesion to samples.

Sample images	
Optical microscope magnification 400x	Fluorescence microscope magnification 400x
Electro -polishing 5minutes	*E.coli* Electro -polishing 5minutes
	
Reference sample	E.coli Reference sample
	
Grinding 10 minutes	*E. colic* Grinding 10 minutes
	
Grinding 15 minutes	*E.coli* Grinding 15 minutes
	

Conclusions

The presented research makes it possible to indicate both the etching and the processing method of 316L steel for further surface modification in terms of its microbiological properties.

References

[1] K. Mydłowska, E. Czerwińska, A. Gilewicz, E. Dobruchowska, E. Jakubczyk, Ł. Szparaga, P. Ceynowa, J. Ratajski: Evolution of Phase Composition and Antibacterial Activity of Zr–C Thin Films. Processes 8(3) (2020) 260. doi:10.3390/pr8030260/
[2] C. Pedrazzini, P. Giordani: „Patent US5843240A”, 1998.

INFLUENCE OF FILTERING MATERIAL ON THE MICROBIOLOGICAL SAFETY OF PROTECTIVE HALF MASKS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 59]

Introduction

Filtering materials in protective half masks should stop microorganisms whose diameter is greater than 0.1 μm. Moreover, they must not restrict the air flow during breathing too much, otherwise asphyxia or anoxemia may occur [1]. The aim of the presented research was to evaluate the microbiological hazards related to the use of protective half masks.

Materials and Methods

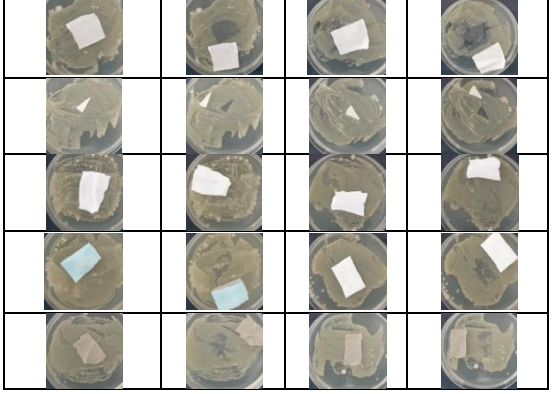
1.Protective half-mask made of a medical fabric composed of 50% cotton, 50% polyester; 2. FS-17V FFP1 NR D half mask (three-layer structure with a welded periphery, needle-punched polyester non-woven fabric, melt-blown polypropylene non-woven fabric. Nose seal made of polyethylene foam); 3. Antibacterial hygienic half mask with silver ions (microfibers with silver ions in phosphate glass); 4. Three-layer surgical half-mask (1st layer of polypropylene non-woven fabric; 2nd layer of melt-blown paper filter; 3rd propylene non-woven fabric. Latex-free) 5. Double-layer cotton half-mask (100% cotton, hand-sewn). The bacteria used in the study came from the American Type Cultures Collection: *Pseudomonas aeruginosa* ATCC 27853.

The evaluation of the antimicrobial activity of the masks was performed using the direct Koch method in accordance with ASTM E2922. The material's susceptibility to adhesion of microorganisms was assessed in accordance with the procedures included in the ISO 22196: 2011 standard with modifications concerning the assessment of the viability of microorganisms. The microbiological cleanliness of the masks used for two hours was assessed by the impression method. The effectiveness of the disinfection methods was assessed for boiling water, water with detergent and alcohol using the Koch method.

Results and Discussion

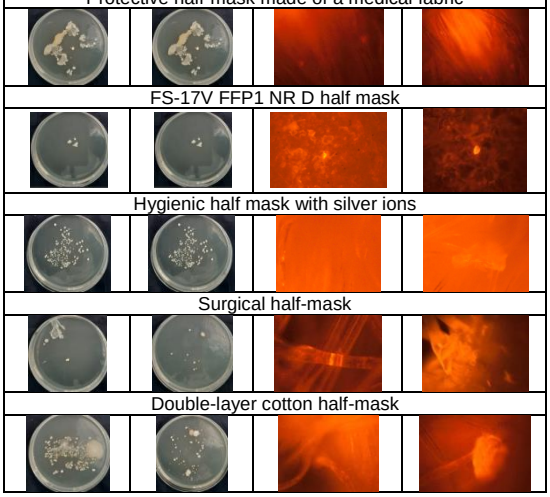
The medical cloth mask and the FFP1 mask filter had the best bacteriostatic effect (TABLE 1).

TABLE 1. Bacteriostaticity of the mask material in relation to Pseudomonas aeruginosa.

The inner part of the mask	The outer part of the mask
	

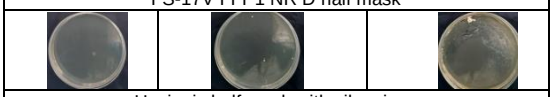
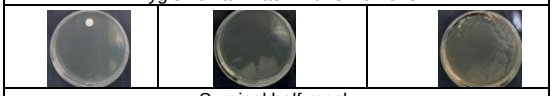
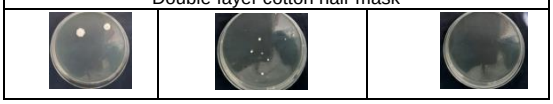
After two hours of use (TABLE 2) the highest number of microorganisms was present on half-mask with silver ions and on the hand-sewn cotton mask.

TABLE 2 Assessment of microbiological purity and adhesion of bacteria after two hours of mask usage.

Assessment of microbiological purity		**Adhesion of bacteria to the mask material**	
Inner part of mask	Outer part of mask	Inner part of mask	Outer part of mask
Protective half-mask made of a medical fabric			
			
FS-17V FFP1 NR D half mask			
Hygienic half mask with silver ions			
Surgical half-mask			
Double-layer cotton half-mask			

Applied methods of masks disinfection (TABLE 3) turned out to be ineffective. The presence of microorganisms was found on each of the tested materials.

TABLE 3. Assessment of the effectiveness of disinfection methods.

Disinfection method		
Boiling water	Water with detergent	Alcohol
Protective half-mask made of a medical fabric		
		
FS-17V FFP1 NR D half mask		
		
Hygienic half mask with silver ions		
		
Surgical half-mask		
		
Double-layer cotton half-mask		
		

Conclusions

The obtained test results indicate that the material from which the masks are made should be static for microorganisms, e.g. in the case of masks made of medical cloth and the FFP1 mask filter. In order to achieve a high safety effect i.e. elimination and reduction of adhering microorganisms disinfection methods for reusable masks should be combined in the following order: dipping in boiling water then washing with detergent and alcohol. Based on the research, it was found that the time of masks usage should be limited to a maximum of two hours.

References

[1] I. Krucińska, W. Strzembosz, K. Majchrzycka, A. Brochocka, K. Sulak: Biodegradable Particle Filtering Half Masks for Respiratory Protection, FIBRES & TEXTILES in Eastern Europe (2012) 20 6B(96) 77-83.

FIRST TESTS OF EXTRUSION PROCESS USING ARTHROSCOPIC 3D BIOPRINTING HANDHELD TOOLS PROTOTYPES

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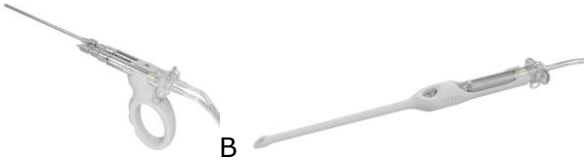
[ENGINEERING OF BIOMATERIALS 158 (2020) 60]

Introduction

Novel nanocomposite responsive materials combined with adipose tissue-derived stem cells (ASCs) and a remotely controllable ultrasound (US) is developed within the H2020 project as the innovative osteoarthritis treatment procedure[1]. The prototype of 3D bioprinting handheld tool[2] was developed as a device which should assist the surgeon in depositing the bio inks during arthroscopy in a well-controlled shape according to the patient's cartilage anatomy. First tests were performed to determine working parameters of extrusion.

Materials and Methods

Hydrogel and ASCs (or ASCc premixed with a hydrogel) delivery – two optional scenarios were considered: single and dual channel tools. Printing resolution for tested materials depends strongly on rheological properties. Suitable extrusion pressure needs to be adjusted and considered for particular extrusion coaxial channel with regard to its length and cross section extrusion area. Five different printing tools have been developed: handheld dual and single channel extruding tool (FIG. 1) – for hydrogel, handheld primer extruding tool and handheld curved spatulas (5º and 20º) to allow spreading or shaping extruded material(s) along cartilage lesions.



A B

FIG. 1. Handheld dual (A) and single (B) channel extrusion tool.

Materials with different density were tested: 0.5% and 1,5% Gellan gum not sterilized and after sterilisation in autoclave, 1.5% Gellan gum + BaTiO3 nanoparticles (1% wt.) not sterilized and after sterilisation in autoclave, collagen from jellyfish – JellaGel, VitroINK 3D (TheWellBioscense: Ref. INK01-2) and VitroINK RGD (TheWellBioscense: Ref. INK02-3).

Pressure value for start of extrusion (kPa) was measured for each material for outer shell (10g) and inner shell (14g) in the dual channel tool. The materials rheological behaviour was observed. Further tests have been done for different needle sizes (gauges) with regard to a liquid material formulation, namely 0.5% Gellan gum (no autoclave). The intention was to observe for which tubing sizes controlled liquid extrusion can be obtained.

Manipulations with the dual and single channel extruding tools in the knee phantom were performed to evaluate the manipulation possibilities and accessibility of particular compartments as well as extrusion conditions.

Results and Discussion

Handheld extruding tools fulfil the following specification: ergonomic and convenient to use; allowing mounting cartridges with materials (biomaterials, primer) – cartridges are visible after mounting to recognize the level of consumed biomaterial.

Extrusion parameters for more dense materials (especially for the outer shell) require pressure values higher than the ones offered by the control unit. The control unit is able to supply the pressure up to 90kPa while VitroINK hydrogels required (for outer shells) 125kPa and 140kPa to initiate the extrusion. Reducing the inner shell diameter to match the desired size for particular material will significantly reduce pressure values for the outer channel thus there is no recommendation to increase the compression values offered by the control unit.

For materials in a liquid form (or less dese gel) for the determined gauges of tubing some uncontrolled extrusion appears after application of minimal value of pressure delivered by the control unit. By decreasing the diameter of the tube a controlled extrusion can be obtained.

The manipulation in the knee phantom has shown that while entering the operating area with a tool, there is a risk that some tissues (e.g., fat, or synovial membrane) might stuck in the tip of the endoscopic tool (FIG. 2).

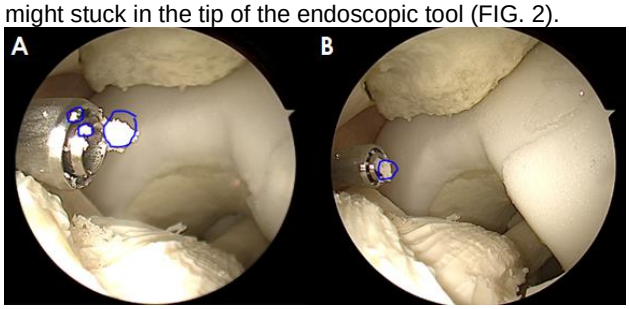


FIG. 2. Possible clogging of tissues at the tip extruding tool.

The possibility to approach the femoral condyle areas from different perspectives and under different angles were tested as well. The tool allows accessing to different parts of the condyle, but the angled approach may not be the most suitable way to extrude biomaterials from the tool. It seems the dual-channel tool may be suitable for perpendicular approach only.

Conclusions

The bioprinting system delivers functionality allowing extrusion of biomaterials. The tubing size of the extruding tool should be adjusted according to the expected density of the biomaterial, which might be: ASC in liquid form and ASC premixed with a hydrogel (in different proportions).

To avoid the problems illustrated in FIG. 2. using arthroscopic cannulas might be recommended. Such approach will reduce the risk of tool passage through tight tissues therefore should be considered in the future.

It is recommended to propose the desired hydrogel material and possible mixing proportions with ASC to perform further tests with different tube sizes and final determination of pressure values. Once the required material is determined this allows also to propose a suitable solution for cross linking strategy.

Acknowledgments

The project leading to the presented results (ADMAIORA) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 814413.

References

[1] https://www.admaiora-project.com/
[2] K.Lenartowicz, T.Gapinski *et al.,* Engineering Of Biomaterials 153 (2019) pp. 44

CARBON-BASED COATINGS ON TITANIUM SUBSTRATE, LASER MODIFED TO CONTROL ENDOTHELIUM CELL GROWTH

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[ENGINEERING OF BIOMATERIALS 158 (2020) 61]

Introduction

Engineering of biomaterials requires a thorough understanding of cell-material interaction. The project concerns the surface modification of the material with its destination in the regeneration of the cardiovascular system. Modulation of surface parameters like topography, chemistry or microstructure have direct influence onto cellular response [1-4]. Nanopatterned surfaces are an effective tool for manipulating the type, number, spacing and distribution of ligands for cell adhesion receptors on the material surface. As a consequence, these surfaces are able to control the size, shape, distribution and maturity of focal adhesion plaques on cells, and thus cell adhesion, proliferation, differentiation and other cell functions. Control of cell phenotype involves a variety of signaling pathways and transcriptional regulators. This multifunctional signaling molecule is part of adhesion contacts in the endothelium and is able to translocate into the nucleus to activate genetic programs and control proliferation and the fate of the cells. Laser interference lithography, consisting in the creation of organized periodical surfaces based on selective material ablation offers the possibility to create 2D and 3D patterns on surfaces. The project concerns a novel approach of the surface modification. The surface modification should give an influence on the micro-vessel formation for the heart endothelium cells.

Materials and Methods

Migration channels were prepared by laser ablation. Thin, nanometric fragments of the coating with a length of 50 nm were removed in half of its thickness. The process of ablation takes place during a laser pulse as a result of interaction of laser radiation (absorption and scattering) with ejected material in liquid form. During the surface treatment of the material with pulsed laser radiation with the density of energy appropriate in time (power density) the following phenomena occur: absorption of radiation and thermal or photochemical effects. The desired reflection requires a low level of radiation. Arousal requires. Electronic transmission microscopy. The analysis of the structure of migration channels was carried out using Transmission Electron Microscopy (TEM).

For TEM analysis thin films were prepared on the cross-section from the migration channel border to the unmodified surface. The influence of nano- and micro patterns on adhesion, directed growth and proliferation of endothelial cells was evaluated. The surface parameters that determine the proper formation of endothelial monolayer and blood vessel formation were characterized.

Results and Discussion

Carbon-based coatings, are among the most promising plasma-based coatings for cardiovascular implants. These are generally characterised by improved haemocompatibility and nontoxicity. Moreover, by controlling the coating deposition parameters and doping, exceptional physicochemical properties of these materials could be modified. Surface modification with laser interference lithography enables controlled cellular growth and growth control (FIG. 1).

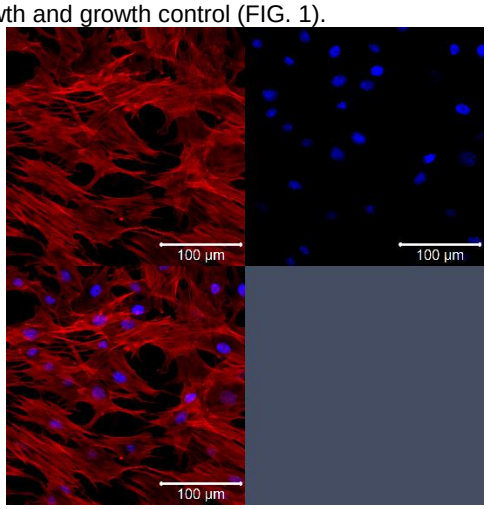

FIG. 1. Endothelium- material interaction controlled by laser modified surfaces.

Conclusions

The control of endothelial cell growth also influences true proliferation and potentiates the formation of vascular-like structures. The formation of proper and dense monolayer endothelial cells on the surface enables effective inhibition of blood clotting.

Acknowledgments

Project supported by:
The research was financially supported by the statue work Z-2 and Project no. 2016/21/N/ST8/00186 "Functional carbon based coatings on titanium substrate, modified by laser ablation designed for the integration with cardiac tissue and ultimately inhibit the blood clotting process" of the National Science Centre Poland.

References

[1] Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. Advanced Drug Delivery Reviews 20011 63:300–311.
[2] Weng Y, Chen J, Tu Q, Li Q, Maitz MF, Huang N. Biomimetic modification of metallic cardiovascular biomaterials: from function mimicking to endothelialization in vivo. Interface Focus 2 (2012) 356–365.
[3] Druecke D, Langer S, Lamme E, Pieper J, Ugarkovic M, Steinau HU, Homann HH. Neovascularization of poly(ether ester) blockcopolymer scaffolds in vivo: long-term investigations using intravital fluorescent microscopy. J. Biomed. Mater. Res. A 2004 68:10–18.
[4] McGuigan AP, Sefton MV. The influence of biomaterials on endothelial cell thrombogenicity. Biomaterials 2007 28:2547–2571.

SURFACE MODIFICATION OF NiTi WIRES FOR ENDOSCOPIC GUIDE WIRES USED IN UROLOGY

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[ENGINEERING OF BIOMATERIALS 158 (2020) 62]

Introduction

The use of NiTi shape memory alloys increases every year [1]. One of the examples of applications are elements of endoscopic devices in the form of guide wires [2]. The core of the guide wire is made of a superelastic NiTi wire coated with a polyurethane polymer. The hydrophilic coverage plays a protective role and makes it easier to overcome tight constrictions in blood vessels or urinary tract.

The problem is the adhesion of polyurethane to the NiTi wire, especially at the tip of the guide wire. Therefore, the aim of the paper was to increase the adhesion of the polymer by increasing the roughness of the wire surface.

Materials and Methods

The subject of the research was guide-wire produced by Endox-Polska Sp. z o. o. The surface of the NiTi wire was etched following conditions shown in TABLE 1. Depending on the effects of etching, the temperature was adjusted from the room temperature (RT) up to 50 °C. The etching time was adjusted in a similar way: from 60 seconds to 15 minutes.

TABLE 1. Composition of etching solutions and etching conditions.

Sample	Solution	Temperature	Time
S1	HCl+HNO_3+ethanol	50 °C	10 min
S2	HCl+$CuCl_2$+ethanol	RT	120 s
S3	HCl+$CuSO_4$+H_2O	RT	120 s
S4		RT	180 s
S5		40 °C	60 s
S6		50 °C	120 s

The surface of the wires was observed with use of light microscope OLYMPUS GX-51. The roughness of the NiTi wires was characterized by the parameters: Ra, Rz, Rz measured on profilometer Mitutoyo Surftest sj-500. Finally, the adhesion of polyurethane to the NiTi surface was determined by the force from the static bursting test using Zwick/Roell testing machine. The maximal force was determined as an average one from 5 measurements.

Results and Discussion

The etching of the wire at room temperature for even 15 minutes in the mixture consisted of HCl + HNO_3 + ethanol did not bring any positive changes in the surface roughness. Raising the temperature of the solution and extending the etching time to 10 minutes resulted in a slightly twofold increase in the Ra parameter in comparison to the initial state (FIG. 1). Changing the etching solution to HCl + $CuCl_2$ + ethanol and carrying out the etching at room temperature for a relatively short time - 120 s resulted in an increase of roughness of 10 times. In consequence of that the increase in the breaking load from 209 N (initial state) to 224 N was observed (FIG. 2).

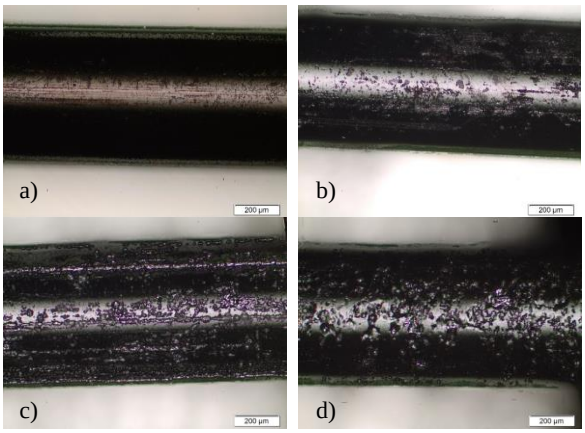

FIG. 1. Images of surface of the NiTi wire at initial state (a), and after etching for sample S1 (b), S2 (c) and S5.

The use of HCl + $CuSO_4$ + H_2O solution and etching at room temperature for 180 s gave a result similar to the previous solution. However, a significant increase in roughness (30 times) and the breaking force (229N) was measured for etching carried out at 40°C with a relatively short time of 60 s. Raising the process temperature to 50°C and extending time to 120 s resulted in an increase of roughness up to 4,5 um. However, the pitting produced on the surface of the NiTi wire lowered the adhesion and the breaking strength was comparable to one received at the initial state.

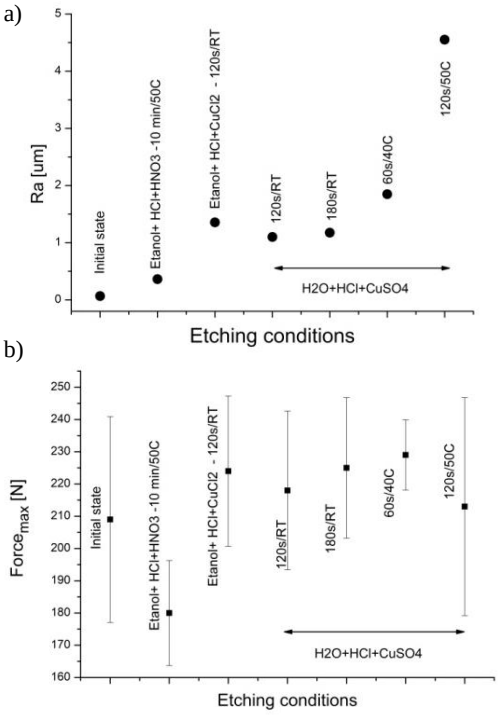

FIG. 2. Arithmetic mean of the roughness profile R_a (a) and maximal force F_{max} (b) versus etching conditions.

Conclusions

The etching conditions carried out in the HCl+$CuSO_4$+H_2O (40°C/60s) or HCl+$CuCl_2$+ethanol (RT/120s) solution significantly increase the adhesion of polyurethane to the NiTi wire surface.

References

[1] T. Yoneyama, S. Miyazaki, Shape memory alloys for biomedical applications, Woodhead Publishing Limited, Cambridge (2009).
[2] L. Yahia, Shape memory implants, Springer-Verlag Berlin Heidelberg (2000).

COMPARATIVE STUDY ON MECHANICAL AND BIOACTIVE PROPERTIES OF DIFFERENT NANOPATTERNED TiO_2 SUBSTRATES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 63]

Introduction

Nanostructured surfaces are considered as very attractive for orthopaedic applications. They are capable of combining different properties, such as high osseointegration with the ability to be used as drug delivery systems. Electrochemical anodization gives the opportunity to produce suitable nanotubular or nanoporous surface structure. Titanium oxide nanotubes (NT) have been extensively investigated for localized controlled release of therapeutics [1], however, their brittle nature could be significantly limiting factor for orthopaedic purpose. In this study, we have produced crystalline nanoporous u-shaped structure (US) of anodized TiO_2 with improved resistance to scratch compared to NT. Also, the US substrate was successfully modified with hydroxyapatite (HAp) coating and investigated for bioactivity.

Materials and Methods

Substrates were prepared on 14x14 mm^2 Ti plates by standard two electrode anodization at room temperature in potentiostatic mode. During the process voltage was kept constant at 50 V. Afterwards, NT and US were annealed in oxidizing atmosphere at 600ºC for 1 h. The US are formed when the NT are removed from the surface and thin layer of TiO_2 is covering patterned substrate. In order to produce HAp coatings, the hydrothermal process was carried out in an autoclave from solution containing: calcium salt hydrate, diammonium phosphate, calcium chelating agent. The morphology and structure of anodized and coated with HAp specimens were characterized using XRD, SEM-EDS and Raman spectroscopy. We evaluated adhesion strength of the nanopatterned samples using combination of SEM, EDS and Nano-Scratch Test System. Bioactivity was examined after 2 weeks of incubation in SBF.

Results and Discussion

SEM images presenting two nanopatterned structures of anodized titanium are shown in FIG. 1. The height of US (left image) is approximately ½ of their diameter, while NT exhibit micrometer-long tubular structures (right image).

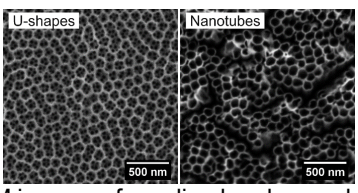

FIG. 1. SEM images of anodized and annealed at 600°C TiO_2 layer in the form of US and NT.

FIG. 2 presents SEM images of sample surfaces after the scratch test, where three main areas are identified: the beginning of scratch (left image); the intermediate area showing deformed but still continuous (according to EDS measurement) layer of the crystalline TiO_2 (middle image), and the area of layer delamination with substrate exposition (right image).

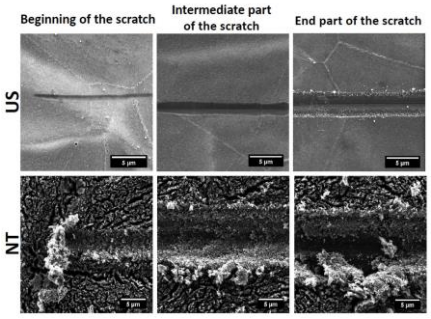

FIG. 2. SEM images showing sample surface of US and NT after the scratch test.

It is very important to consider, that at the time of implantation, orthopaedic implants are subjected to considerable mechanical stress. Most studies on implants with nanoscaled topography focus on their biological properties, however only few scientific reports consider investigating mechanical properties of TiO_2 nanotubes, especially regarding resistance to scratch [2]. In our opinion, nanoscaled topography requires more sophisticated approach than determining adhesion strength of nanopatterned layer only from acoustic emission examination, friction measurements or optical microscopy assessment. A deeper insight into evaluating the resistance against scratch of nanotextured surfaces can be achieved by combining the nano-scratch test with SEM imaging. During electrochemical growth of TiO_2 NT beneath the nanotubular layer a barrier layer of continuous oxide film is formed at the oxide/metal substrate interface [3]. Until now, none of the studies on adhesion considered the importance of this barrier layer, and thus determining the critical load at which delamination of the thorough oxide layer occurs (NT layer along with barrier layer). Using SEM imaging, it is possible to evaluate with good precision surface damage of the nanotubular structure from normal-load lateral scratches, however it is difficult to identify when the continuous oxide barrier layer starts to delaminate. For this purpose, we performed simultaneous SEM and EDS measurements after the scratch test, to investigate when TiO_2 layer experienced a complete delamination (i.e. NT/US layer along with barrier layer) with exposure of the metallic substrate. The determined critical normal with this method loads were of 22.0(3) mN for US and 11.0(3) mN for NT.

Functionalization of the US substrate with hydroxyapatite coating (HApUS) under hydrothermal conditions resulted in high bioactivity after 2 weeks of immersion in SBF.

Conclusions

In this study, we present crystalline nanoporous u-shaped structure of anodized titanium with twice higher resistance to scratch in comparison to brittle NT. We consider that this substrate could be an alternative material to nanotubes suitable as smart drug delivery platform. The HApUS coating may improve the currently used titanium based prosthesis.

Acknowledgments

This study was partially supported by NCN, grant UMO-2015/19/D/ST3/01843.

References

[1] W. F. Oliveira, I. R. S. Arruda, G. M. M. Silva, G. Machado, L. C. B. B. Coelho, and M. T. S. Correia, *Mater. Sci. Eng. C* 81 (2017) 597–606.
[2] M. Sarraf *et al.*, *Process. Appl. Ceram.*, 11 (2017) 311–321.
[3] P. Acevedo-Peña, L. Lartundo-Rojas, and I. González, *J. Solid State Electrochem.*, 17 (2013) 2939–2947.

EVALUATION OF N,N-DIMETHYLACETAMIDE LEVEL IN POLYURETHANE IMPLANT ELEMENTS MANUFACTURED IN DIP-MOLDING PROCESS

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[ENGINEERING OF BIOMATERIALS 158 (2020) 64]

Introduction

Polyurethanes are the excellent biomaterials used in many medical applications, particularly in cardiovascular implants, such as artificial heart diaphragms, vascular grafts etc. ChronoFlex AR/LT (prod. AdvanSource Biomaterials, USA) are polycarbonate urethanes designed for dip-moulding applications [1] and have confirmed biocompatibility properties. These unique materials are fully synthesized in solvent such as N,N dimetyloacetamid (DMAc). The thin membranes are manufactured within the dip-moulding process, as the part of long-term implant device. The level of DMAc concentration in the final implant element is one of the technological and biological problem, which has to be solved for clinical application. The final product must be biocompatible and the maximum level of residual DMAC acceptance is: 1090 ppm [2].

Materials and Methods

The aim of the study was the DMAc level concentration measurements in polyurethane Chronoflex AR/LT thin elements manufactured in the dip-moulding process in Artificial Heart Laboratory.

The DMAc residual concentration examination in polyurethane samples collected form final implant elements, was performed by gas chromatography analysis. The samples were selected from the different areas of implant element (membrane elements with different surface topography and thickness, n=6 for each area). The measurements were performed for classical and modified polyurethane elements washing technology made after dip-moulding process. The DMAc content was analysed after polyurethane samples extraction in water. Calibration experiments were performed using a number of samples basing on the sample enrichment method. Measurements were performed for samples with a known amount of the substance added (DMAc) as well as for research samples.

The polyurethane sample (approximately 0.2g) was placed into 20µl of water (measuring sample) for DMAc extraction. The extracted DMAC was analysed utilizing: PerkinElmer Clarus 500 gas chromatograph with chromatographic column HP-INNOWAX (30cmx0.53mm x1µm).

Results and Discussion

The DMAc level obtained in the elements manufactured with the modified washing technology is presented in TABLE 1.

The DMAc residual content in the thin elements manufactured in the dip-moulding process depends on samples thickness and surface topography. DMAc level for samples taken from the “mirror” (thickness 0,32mm) ranges from 200ppm to 330ppm; for samples taken from the “sphere” (thickness: 0,35mm) ranges from 120ppm to 280ppm and for samples taken from the “ring” (thickness 0,38 mm) ranges from 410 ppm to 990 ppm. The study shows that the highest concentration of DMAc is in samples taken from the ring (the thickest area of implant element manufactured within the injection process). However, the measured values qualifies the whole product (implant element) as conform and safe in the aspect of biocompatibility connected with the maximum accepted level of DMAc.

TABLE 1. DMAc content level.

No.	DMAc cont. [ppm] in mirror	DMAc cont. [ppm] in sphere	DMAc cont. [ppm] in ring
1	220	120	460
2	220	130	410
3	220	140	600
4	280	280	990
5	200	130	410
6	330	170	490

Conclusions

DMAc residual content analysis in the elements manufactured with Chronoflex AR/LT in the dip-moulding process, has shown that the solvent content does not exceed the required level of 1090 ppm, staying on the level about from 10% to 60% of maximum accepted value. The tests have confirmed the Chronoflex AR/LT elements utilisation in the long-term implants.

Acknowledgments

Tests performed within the commercial project.

References

[1] AdvanSource Biomaterials http://www.advbiomaterials.com/products/polycarbonate/chronoflex_ar.html Accessed 30 August, 2020

[2] European Medicines Agency ICH Q3C(R5) Impurities: Guideline for Residual Solvents. Step 4. London, UK: EMA; February 4, 2011. Available at: https://www.tga.gov.au/sites/default/files/ichq3cr5.pdf. Accessed 30 August, 2020

DEGRADATION STUDIES OF POLY(DIOL CITRATES) FOR VASCULAR TISSUE ENGINEERING PURPOSES

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[ENGINEERING OF BIOMATERIALS 158 (2020) 65]

Introduction

Poly(diol citrates) (PDC) have been recognised as potential materials for tissue engineering of blood vessels with diameter <6 mm, due to their biodegradability, cytocompatibility, bioactivity and their capability to be modified in many ways [1,2]. The aim of this study was to evaluate degradation kinetics and mechanism of two types of PDCs obtained via polycondensation and modified or not with panthenol or glutathione.

Materials and Methods

Cross-linked poly(hexamethylene citrate) and poly(octamethylene citrate) (cPHC and cPOC, respectively) were obtained. In brief, citric acid and 1,6-hexanediol/1,8-octanediol in molar ratio 2:3 were melted together at 140°C for 40 min under stirring to synthesise a prepolymer, which was then dissolved in 96% ethanol, precipitated in distilled water, lyophilised (0.37 Ba) and dissolved again in 96% ethanol. Then either panthenol or glutathione (concentrations 0%, 0.4% and 0.8%) were added to the prepolymer and left for post-polymerisation for 10 days at 80°C under vacuum (200 mbar). Round samples (diameter 8 mm) were excised by a hole punch, weighed and incubated in ultra-pure water (UHQ-water – 10 ml/sample, 37°C) for predefined time intervals (up to 3 months) – 3 samples per time interval and material. After incubation, the samples were weighed with analytical balance in a wet state and once again after lyophilisation to evaluate weight loss and water absorption capacity. Shore hardness tests were also conducted to assess decrease in cross-linking density. Moreover, incubation fluid was characterised by pH measurement to further evaluate the degradation progress.

Results and Discussion

Weight loss of the samples was mostly dependent on the type of diol (FIG. 1) – cPHC more hydrophilic alkylene units degraded faster (15-25% weight loss) as compared to cPOC (4-9% weight loss) after 3 months. The influence of the additives used on the weight loss of studied samples was less significant – samples modified with panthenol were degrading slightly slower while the ones modified with glutathione degraded faster than the controls. In all cases notable weight loss became appeared between day 21 and 35 of the experiment. This observation was found in line with decrease in hardness and increase in susceptibility to water absorption. The pH of the degradation fluid was decreasing mostly during the first days of incubation to the values of 3.5 – 4.5 and 4.5 – 5.5 for cPHC and cPOC materials, respectively.

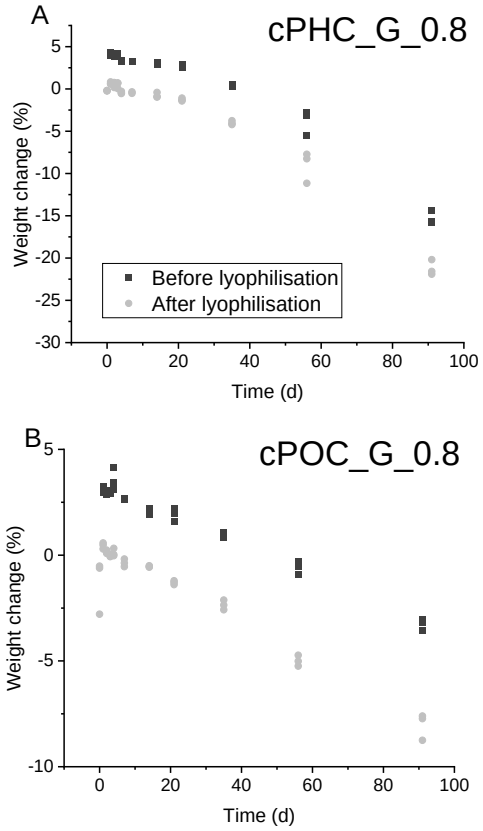

FIG. 1. Weight change for cPHC and cPOC with addition of 0.8% of glutathione. Difference in degradation rate between both polymers is observed.

The results indicate, that the initial increase in samples weight is due to water absorption. It suggests that water molecules enter the structure and expand free spaces between polymer chains. Thus, in the course of hydrolysis of ester bonds the number of cross-links between polymer chains decreases, resulting in lower hardness and higher water absorption. pH changes observed prior to weight loss suggest elution of unreacted monomers. The results show that degradation is gradual and slow. Acidic products of degradation should not inhibit surrounding tissue regeneration, as *in vivo* they will be released to the flowing blood, which has a very good buffering capacity.

Conclusions

We evaluated degradation kinetics of cPHC and cPOC materials, that is rather influenced by the type of diol and post-polymerisation time, as compared to other studies [2], than by the type of a modifier. Slow and gradual degradation creates a good perspective for vascular prostheses, that will not lose their mechanical properties before the native tissue is rebuilt. Obtained materials seem to be promising for vascular tissue engineering purposes.

Acknowledgments

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References

[1] R. T. Tran, J. Yang, G. A. Ameer, „Citrate-Based Biomaterials and Their Applications in Regenerative Engineering”, *Ann. Rev. Mater. Res.*, 45 (1), 2015, 277–310
[2] J. Yang, A. R. Webb, G. A. Ameer, „Novel Citric Acid-Based Biodegradable Elastomers for Tissue Engineering”, *Adv. Mater.*, 16 (6), 2004, 511–516

HIGHLY POROUS POLURETHANE-BASED GRADIENT SCAFFOLDS FOR TISSUE ENGINEERING OF OSTEOCHONDRAL DEFECTS

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Introduction

Polyurethanes (PU) are known for their versatility and diversity of properties that can be tailored to a specific application by playing with PU building blocks and chemical composition. They can be volume- or surface-modified to create polymer-matrix composite systems with improved properties. Also, studies have shown that introduction of chitosan, a natural polysaccharide, can positively affect biocompatibility of PU. Bearing in mind that polyurethanes are generally known for their superior (when compared especially to natural polymers) mechanical properties and susceptibility to various modifications, including these responsible for enhanced bioactivity/biocompatibility, one can safely state that their potential for application as biomaterials is extensive. Among many others, they can be used as scaffolds in regeneration of osteochondral defects. Tissue engineering applications are demanding in many ways and impose certain material- and scaffold-related criteria, like biodegradability, nontoxicity or high porosity to name a few. In this study, a series of highly-porous polyurethane-based composite scaffolds modified with chemically-grafted hydroxyapatite (HAp) and graphene oxide (GO) was developed and characterized.

Materials and Methods

Highly porous polyurethane-based scaffolds were obtained in a one-step bulk polymerization method. First, dry poly(ethylene glycol) (PEG, M_w=2000 g/mol and poly(ε-caprolactone) diol (PCL, M_w=2000 g/mol) with a molar ratio of 1:3 were melted under 60°C, followed by addition of a medical-grade chitosan (CS, DDA = 85%, HMC+). All the reagents were heated. When the reaction system reached 90°C, a chain extender, 1,4-butanediol (BDO), was added and finally, melted 4,4'-diphenylmethane diisocyanate (MDI) was injected under 60°C. The system was mixed thoroughly, left for approx. 4 h in 80°C and subsequently for 12 h in 80°C (120°C for GO samples). The synthesis was carried out under nitrogen atmosphere. For PU-based composites, HAp or GO were dispersed through sonication in the melted polyols. The obtained samples were as follows: PU1 (ref sample), PU2 – PU4 with added 1, 5, and 10% of HAp, respectively, PU5 and PU6 with 0.1 and 1% of GO, respectively. Gradient scaffolds were obtained by gradual synthesis of different PU on top of each other. Physicochemical properties of the materials were examined using various methods.

Results and Discussion

As shown in FIG. 1, all of the non-modified and HAp-modified PU were highly porous (PU1-4). Microstructure of the GO-modified samples was more dense, but still with open porosity. With higher GO content (0.1% vs 1%) some nonhomogeneity was observed.

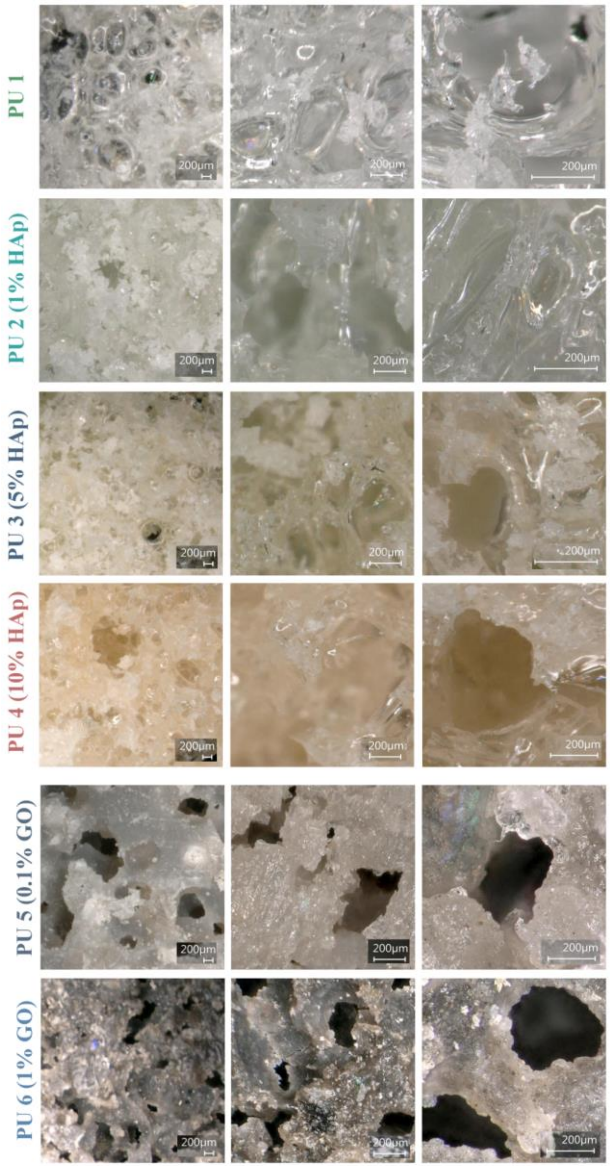

FIG. 1. Digital microscope images of the cross-section of the samples.

Improved mechanical properties (compressive strength and Young's modulus) were observed for samples modified with HAp and GO (especially for samples PU4 and PU5, respectively. Addition of HAp positively affected also wettability of the materials. After incubation in simulated body fluid (2 and 4 weeks, 37°C), apatite-like structures were formed on the surface of all the samples. Gradient scaffolds had three distinct zones designed for to fit the osteochondral defects requirement: (i) thin layer (approx. 3 mm) of PU/GO, non-modified intermediate zone (as PU1) and HAp-rich lower zone destined for subchondral bone area.

Conclusions

Polysaccharide (chitosan) containing polyurethanes were successfully synthesized. They were further modified with hydroxyapatite and graphene oxide. It was possible to obtain gradient scaffolds for soteochondral defects regeneration.

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