RIGA TECHNICAL UNIVERSITY
Faculty of Materials Science and Applied Chemistry
Institute of General Chemical Engineering

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Student of the Doctoral Study Programme "Materials Science"

PREPARATION AND CHARACTERISATION OF BIOMATERIALS BASED ON TITANIUM DIOXIDE CERAMICS

Summary of the Doctoral Thesis

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Professor, *Dr. sc. ing.*
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THE DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF ENGINEERING SCIENCES

To be granted the scientific degree of Doctor of Engineering Sciences, the Doctoral Thesis will be defended on 14 December 2016 at the Faculty of Materials Science and Applied Chemistry of Riga Technical University, 3 Paula Valdena Street, Room 272.

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I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Engineering Sciences is my own and does not contain any unacknowledged material from any source. I confirm that this Thesis has not been submitted to any other university for the promotion to other scientific degree.

Inga Narkevica ……

Date: ……

The Doctoral Thesis has been written in Latvian. It contains Introduction, 3 Chapters, Conclusions, References with 193 information sources and Appendices. The volume of the Doctoral Thesis is 162 pages. It has been illustrated by 104 figures and 24 tables.

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Inga Narkevica

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GENERAL OVERVIEW OF THE DOCTORAL THESIS

Introduction

Nowadays there is an increasing need for a variety of orthopaedic implants for repair of joint and bone fractures, restoration of osteoporosis or tumour damaged bones. Statistics shows that every year 15 million bone fractures take place, and 10 % of bone fractures do not grow together. Consequently, each year the requirements for bone replacing materials become increases, for example, they must not only be bioactive, which would facilitate the apatite formation on its surface, but also bone scaffolds should meet certain criteria, including porosity, pore size and mechanical properties similar to those of the damaged area of bone. Special attention in bone tissue engineering is devoted to 3D porous scaffolds with fully open and interconnected pore structure, as they can promote vascularisation, bone cell adhesion and proliferation that further enhance new bone formation. One of biocompatible materials that could be used for bone tissue regeneration is porous titanium dioxide (TiO_2) ceramics, which shows higher mechanical properties compared to other porous ceramic materials and osteoconductivity.

Many studies have been performed to improve bioactivity of TiO_2 nanoparticles (1D) and coatings (2D), but there is a lack of information about production and characterisation of bioactive and 3D highly porous TiO_2 scaffolds. In recent years, the emphasis has been put on application of electrical stimuli in bone defects in order to promote bone regeneration. Studies have shown that bone forming cell or osteoblast activity – migration to bone defect site, attachment to the surface and proliferation – is enhanced using electrical stimuli. These findings have led to the development of materials, which are able to locally deliver electrical current in bone defects and promote bone regeneration. Use of electrically conductive and porous ceramic scaffolds in order to supply electrical stimuli has not been mentioned in the literature. Therefore, the experimental work is devoted to the preparation of highly porous TiO_2 ceramic scaffolds with desirable implant material characteristics, for example, porosity, pore size and mechanical properties, and in the next step modifying them to obtain bioactive or electrically conductive scaffolds. 3D porous and electrically active TiO_2 scaffolds can also be used in electrophoretic deposition as a substrate, for which data in the scientific literature is impossible to find.

Aim of the Doctoral Thesis

To obtain highly porous scaffolds based on titanium dioxide ceramics for bone tissue regeneration using polymer replica method, analyse properties of the obtained ceramics and evaluate possibilities of scaffold modification.

Tasks of the Doctoral Thesis:

1. To summarise the information found in the scientific literature about bone tissue regeneration, important biomaterial characteristics, fabrication methods of porous ceramics, and properties and application of TiO_2 ceramics.
2. To evaluate impact of used TiO_2 powder and subsequent thermal treatment on physical properties, bioactivity, protein sorption and bacteriostatic effect of obtained ceramics.
3. To develop a methodology in order to obtain highly porous and bioactive scaffolds based on TiO_2 ceramics and to assess their physical properties;

4. To obtain electrically active, porous TiO_{2-x} ceramic scaffolds and to assess their physical properties;
5. To obtain polymer or polymer/HAp coating on TiO_2 scaffolds via a vacuum impregnation method and analyse impact of the coating on mechanical properties, *in vitro* bioactivity and degradation of obtained scaffolds.
6. To evaluate cytocompatibility of obtained scaffolds using osteoblast and fibroblast cell lines.

Scientific Novelty of the Doctoral Thesis

Systematic studies about impact of TiO_2 particle size and subsequent post-processing on the physical characteristics, surface properties, *in vitro* bioactivity and bacteriostatic properties of obtained ceramics have been performed.

For the first time, 3D highly porous, cytocompatible and electrically conductive TiO_{2-x} ceramic scaffolds have been produced that hold potential to be used as an electrical stimuli supplier in bone tissue regeneration. Such a material can also be used as an electrode/substrate in electrophoretic deposition.

For the first time, hydroxyapatite/poly(vinyl alcohol) composite coated TiO_2 ceramic scaffolds with improved mechanical properties and *in vitro* bioactivity that can also be used as a local drug delivery system have been obtained.

Practical Significance

Methodology of production and modification of highly porous TiO_2 ceramic scaffolds has been developed that can be potentially used in bone regeneration, providing the desired mechanical strength, open pore structure and bioactivity/ cytocompatibility.

Thesis Statements to Be Defended

1. *In vitro* bioactivity of TiO_2 ceramics or ability of the material to form biomimetic apatite on its surface that is similar in structure and composition to biological apatite found in bones is affected by thermal treatment conditions, during which surface morphology and surface properties of obtained ceramics change.
2. Degree of porosity and mechanical properties of 3D highly porous TiO_2 ceramic scaffolds obtained via a polymer replica method are affected by slurry production technology and thermal treatment. *In vitro* bioactivity of porous scaffolds can be improved by coating with nanosized TiO_2 or hydroxyapatite/poly(vinyl alcohol) composite.
3. Using vacuum heat treatment, it is possible to obtain electrically active and highly porous TiO_{2-x} ceramic scaffolds that can be used as a substrate in electrophoretic deposition.

Approbation of the Results

The scientific results of the research have been summarised in 12 full text scientific articles (10 of them indexed in SCOPUS database) and presented at 21 international conferences.

LITERATURE REVIEW

The literature review summarises information about bone tissue engineering and bone remodelling process, biomaterials used in bone tissue engineering, preparation of highly porous ceramics, TiO_2 properties and application, as well as biomaterial interaction with the surrounding environment in the body.

Musculoskeletal conditions are very common, and thus there is an increasing need for a variety of orthopaedic implants to restore tissues after bone and joint fractures, osteoporosis and bone tumours [1], [2]. One of the ways to restore damaged bone is to use highly porous ceramics as 3D scaffolds because it can serve as a matrix that can be loaded with cells and/or growth factors, thus ensuring the necessary microenvironment for tissue regeneration [3]–[5]. Blood vessels provide all the necessary conditions for bone cell growth. Thus, formation of new blood vessels from pre-existing blood vessels or angiogenesis plays a crucial role in bone tissue regeneration [6], [7]. Very important factor that will affect bone tissue regeneration is porosity and pore size of the scaffold. Pore sizes above 100 µm promote ingrowth of new bone tissues and vascularization, but smaller size pores (below 20 µm) enhance the attachment of bone forming cells and proteins [8].

Developing the implant material, it is necessary to take into account for which bone replacement it will be used. Bones can be dived into compact or cortical bones and trabecular or cancellous bones. Mechanical properties of bone depend on its structural characteristics, for example, porosity (trabecular or cortical bone), mineralization degree, orientation of collagen fibres, *etc*. Literature survey indicates different values of bone mechanical properties, and compressive strength of trabecular bone varies from 0.1 MPa to 16 MPa. It is preferable that the implant characteristics are as close as possible to the natural bone.

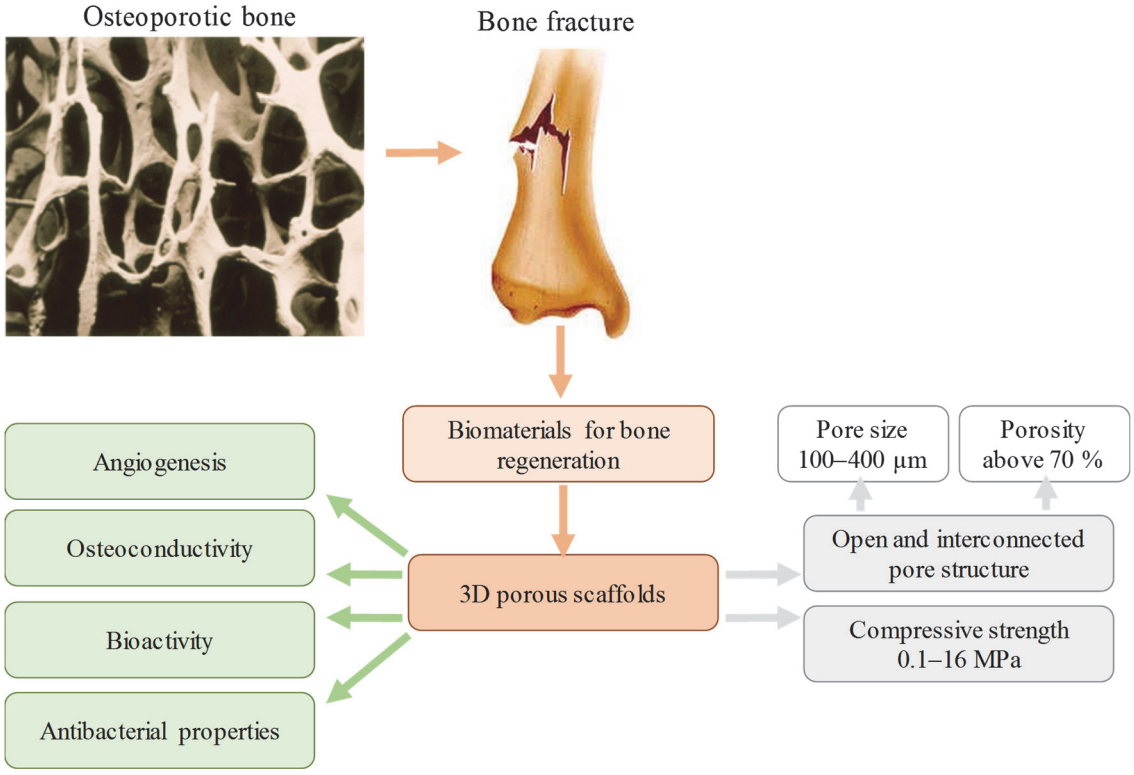


Fig. 1. Schematic representation of the main characteristics of porous scaffolds used in bone tissue regeneration.

Implant materials depending on their interaction with surrounding tissues can be classified: biotolerant, bioinert, bioactive and bioresorbable. Nowadays, increasing attention is dedicated to bioactive materials because they can provide the biological link between the material and the surrounding tissue, thus promoting new bone formation. Biomaterials used in porous scaffold production can be divided into metals, polymers and ceramics. Bioceramic materials have been used for bone replacement for a long time and they can be divided according to different generation. Particular attention is drawn to the third generation bioceramics that is based on the use of the above-mentioned porous material or scaffold for bone regeneration. Nowadays, intensive work is devoted to the development of biocompatible and bioactive, 3D porous scaffolds with the necessary micro and macro porosity and suitable mechanical properties. At the same time, these scaffolds should promote osteoconductivity because it will ensure implant and surrounding bone biological linkage and new bone ingrowth [8]. Most important characteristics of implant material used in bone tissue regeneration that are mentioned in the literature are summarised in Fig. 1.

Different methods are developed to produce porous materials. Depending on the used method, changes occur in porosity, pore size and morphology of the material. Porous precursor impregnation method, known also as a replica method, is one of the first macroporous material production technologies, which is also one of the most common methods mentioned in the literature of highly porous TiO_2 ceramic preparation [9]. TiO_2 ceramics is one of the materials used for production of porous scaffolds because it is possible to obtain more mechanically resistant ceramic scaffold, comparing, for example, to hydroxyapatite ceramics, and it can be easily modified obtaining different advantageous properties associated with TiO_2 structure.

According to the literature review, many studies have been performed on bioactivity improvement of TiO_2 nanoparticles (1D) and coatings (2D), but there is no information about preparation and characterisation of bioactive and 3D highly porous TiO_2 ceramic scaffolds. Thus, the experimental work is devoted to the development of highly porous TiO_2 ceramic scaffolds with desirable implant characteristics, such as porosity, pore size and mechanical properties, and further modifying prepared scaffolds using different methods.

MATERIALS AND METHODS

Schematic summary of the experimental work of the Doctoral Thesis is shown in Fig. 2. TiO_2 anatase powders with average particle size of 15 nm ($nTiO_2$) or 180 nm ($mTiO_2$) were used as raw materials for production of ceramics. Dense TiO_2 ceramics was prepared via compaction or extrusion and was used to analyse physical characteristics (sintering process, phase transformation, density, porosity, electrical, mechanical and surface properties) and *in vitro* properties (bioactivity and bactericidal adhesion/colonization) of obtained ceramics. Protein sorption studies were performed on thermally treated $nTiO_2$ powders. Porous TiO_2 ceramic scaffolds were produced via a polymer replica method (Fig. 3) as a precursor using elastic polyurethane foams and realizing thermal treatment in two stages – burnout of PU foam and high temperature sintering.

Different thermal treatment regimes, temperatures and holding times were realized depending on the used TiO_2 powder, sample production technology and investigated properties.

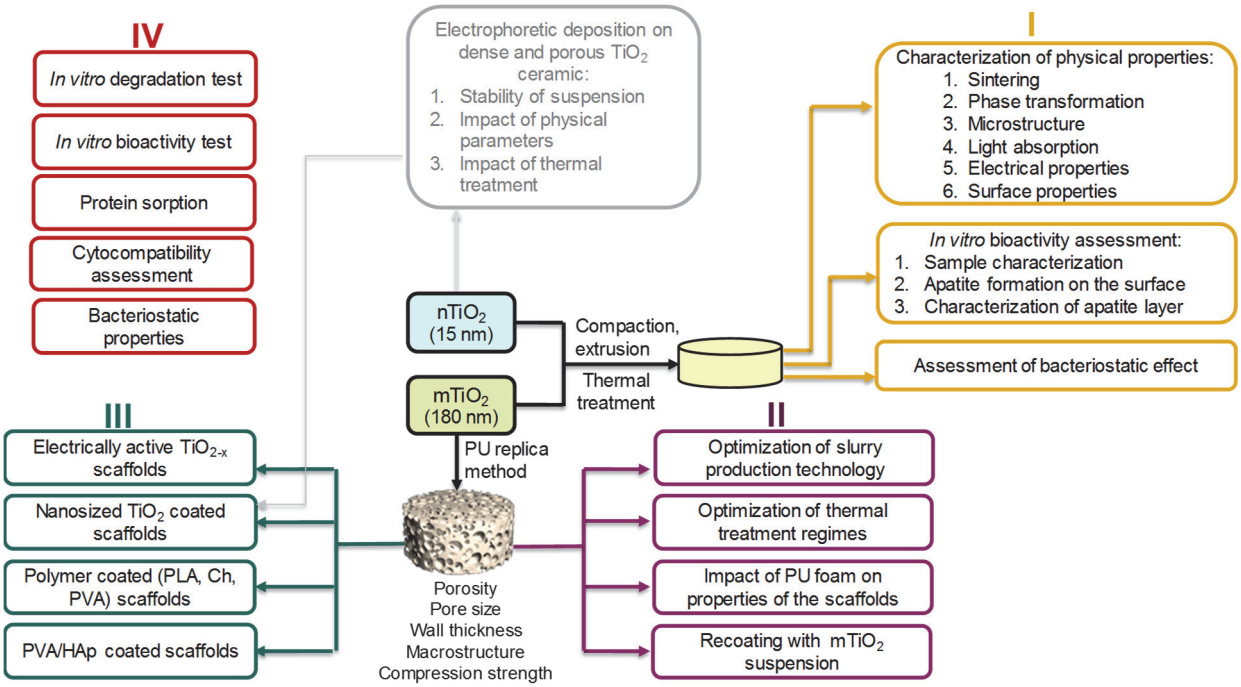


Fig. 2. Schematic summary of the experimental work of the Doctoral Thesis.

Methodological part describes preparation of coated porous scaffolds. Polymers (PLA, Ch or PVA), PVA/HAp and TiO_2 nanoparticle coatings were applied by a vacuum impregnation method followed by blowing with compressed air. The other method used to obtain TiO_2 nanoparticle coating was electrophoretic deposition, where TiO_{2-x} ceramic electrodes served as substrates.

Methods and equipment used to characterise the obtained samples are listed below. High temperature microscope was used to study the sintering process of TiO_2 powders. X-ray diffractometer, Fourier transform infrared spectroscope and *Raman* spectroscope were used for characterisation of structure and phase transformation of the samples. Light absorbance of TiO_2 ceramics was determined using diffuse reflectance spectroscopy. Specific surface area of the samples was evaluated using the BET method. Surface properties of TiO_2 ceramics (surface energy, polar and disperse component, contact angle) were examined using an optical contact angle measurement device. Microstructure of the obtained ceramics was evaluated using a scanning electron microscope and digital microscope. Particle size distribution of TiO_2 slurry used to obtain porous ceramics was determined using a laser diffraction

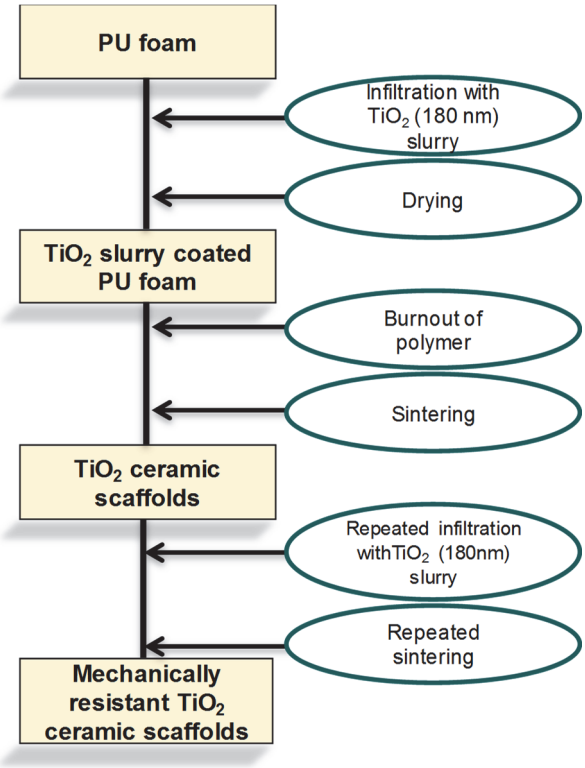


Fig. 3. Flowchart of porous TiO_2 ceramic scaffold production.

method, but rheological properties were measured using a rheometer and calculating the *Casson* viscosity and yield stress via the *Casson* model.

Density, porosity, electrical and mechanical properties were determined for dense and porous TiO_2 ceramics. Porosity and density of dense ceramics were measured using Archimedes' method, but porosity of porous ceramic was calculated by a geometric method. Mechanical properties of dense ceramics were determined using a four-point bending test and of porous ceramics – by means of a compression test.

In vitro studies were performed to analyse the impact of TiO_2 ceramic manufacturing conditions on protein sorption, *in vitro* bioactivity, bactericidal adhesion/colonization and cell (osteoblast and fibroblast) adhesion/proliferation.

RESULTS AND DISCUSSION

1. Characterisation of TiO_2 Ceramic Structure and Properties

Impact of Thermal Treatment on Physical Properties

Impact of $nTiO_2$ and $mTiO_2$ processing and thermal treatment on the sintering process, phase transformation, density, porosity, microstructure, light absorbance, electrical, mechanical and surface properties of obtained ceramics was evaluated.

Powder sintering is one of the most important processes in the fabrication of ceramic material. During sintering the product formed from the powder turns into a compact body. Dilatometric curves of the $nTiO_2$ and $mTiO_2$ powders show significant differences in the sintering process (Fig. 4). $nTiO_2$ powder sintering begins around 550 °C, but in the case of $mTiO_2$ – around 950 °C. XRD data revealed that during the sintering process the phase transformation occurs from anatase to rutile crystallographic modification. Rutile content in the phase transformation interval was calculated from anatase 101 and rutile 110 peaks in XRD pattern and is shown in Fig. 5. In the case of nanopowder phase, transformation occurs at lower temperatures, which can be attributed to smaller particle size and higher specific surface area of $nTiO_2$ powder ($mTiO_2$ ~12 m^2/g and $nTiO_2$ ~150 m^2/g).

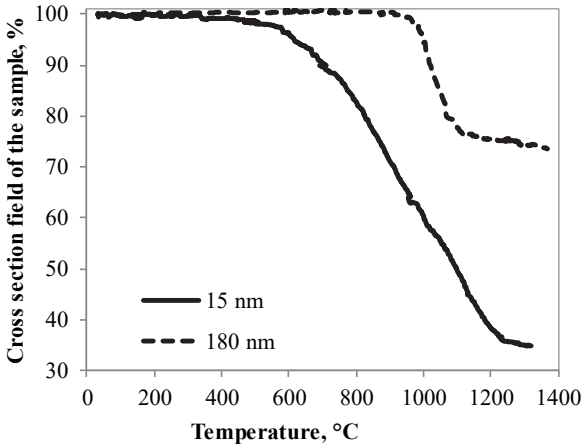


Fig. 4. Dilatometric curves of TiO_2 powders.

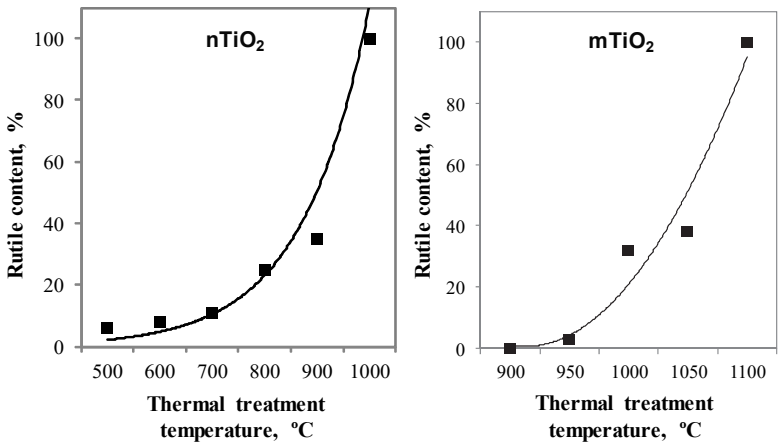


Fig. 5. Rutile content in the samples depending on thermal treatment temperature.

By increasing the sintering temperature, density and grain size of obtained ceramics also increase. High density ceramics (~93 % of rutile theoretical density) in the case of $mTiO_2$ can be obtained thermally treating at 1450 °C that can be characterised with micron-sized grains (above 20 μm) (Fig. 6). In the case of $nTiO_2$, such density ceramics with much smaller grain size (below 300 nm) can be obtained at 1000 °C.

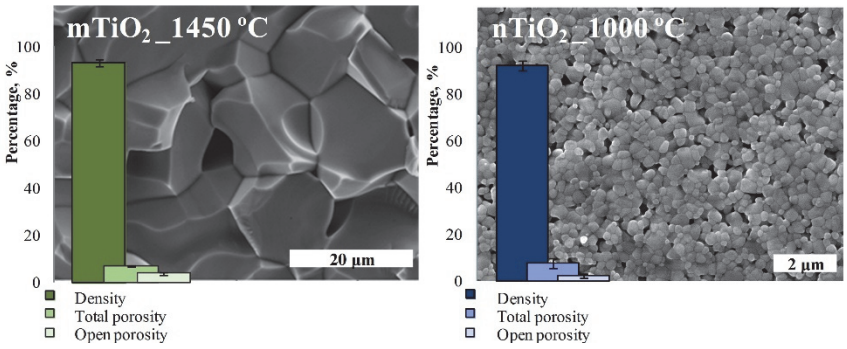


Fig. 6. Microstructure and density of TiO_2 ceramics depending on the used powder and thermal treatment temperature.

Surface properties of biomaterials used in bone tissue repair are very important because they affect interaction with the surrounding environment in the body, for example, protein sorption, bioactivity and bacteria attachment. Pressed $mTiO_2$ samples thermally treated at 1100 °C, 1250 °C, 1350 °C and 1450 °C were used to analyse impact of thermal treatment on the surface energy and wetting angle of obtained ceramics. For comparison, $nTiO_2$ samples thermally treated at 1000 °C were used. Influence of additional thermal treatment under high vacuum conditions or UV-light irradiation on the TiO_2 surface properties was evaluated. Surface energy as well as disperse and polar component of the samples was calculated based on contact angle measurements. Total surface energy of ceramic samples tends to decrease with an increase in the sintering temperature (Fig. 7). It is related to the fact that the system tries to reduce surface free energy during the sintering process. Additional heat treatment under high vacuum conditions does not cause significant changes in the surface energy. Comparing the same density ceramics ($nTiO_2$ – 1000 °C and $mTiO_2$ – 1450 °C), higher surface energy was observed for $nTiO_2$ samples that indicated the effect of surface morphology on surface energy, e.g., nanosized grains increased it. UV-light irradiation drastically increased surface energy of the samples. Wetting angle Θ of the studied samples was below 90°, which meant that TiO_2 ceramics had the hydrophilic surface.

The number of hydroxyl groups on the surface can be determined qualitatively using FT-IR spectra and quantitatively by substituting –OH groups on the TiO_2 ceramic surface with Zn^{2+} ions that are determined using atomic absorbance spectroscopy. Results indicate that increasing the sintering temperature from 700 °C to 1000 °C, the number of –OH groups on $nTiO_2$ ceramic surface decreases. These finding directly correlate to surface energy measurements.

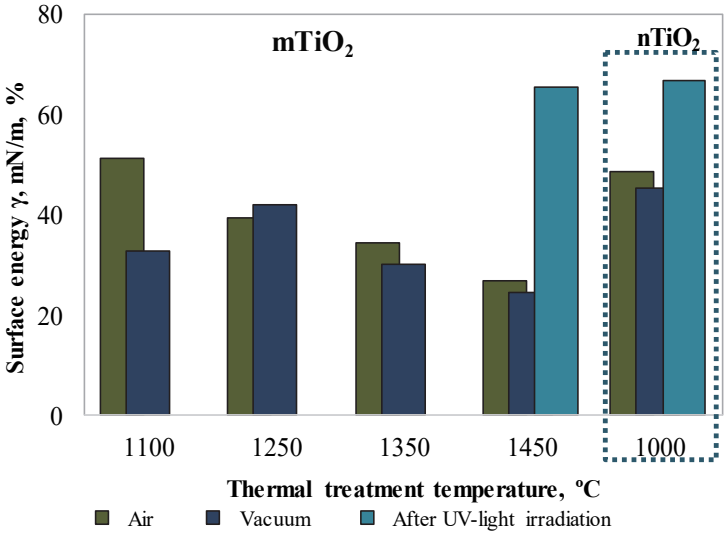


Fig. 7. Total surface energy γ of TiO_2 ceramics depending on the manufacturing conditions.

In Vitro Studies of TiO_2 Ceramics

Assessment of *in vitro* properties is very important to predict biomaterial interaction with the surrounding environment in the body and it helps choose the most appropriate processing conditions to improve the biological interaction with tissues.

Protein sorption. Biomaterials implanted in the living organism firstly come into contact with blood plasma and proteins that exist in plasma. Adsorption of specific proteins on biomaterial surface ensures cell attachment that further enhances bone tissue regeneration at bone defect site [10]. Protein sorption studies were performed using thermally treated (700 °C and 1000 °C temperature) $nTiO_2$ powders and studying adsorption kinetics of bovine serum albumin, as well as acquiring sorption isotherms.

Higher protein sorption exhibited samples that were thermally treated at 700 °C (Fig. 8). Such powder has a much higher specific surface area (700 °C – 37.1 m^2/g, 1000 °C – 3.4 m^2/g), higher surface energy and more –OH groups on the surface. Results indicate that these factors improve protein sorption. Sorption process can be characterised with Langmuir isotherm (Fig. 8), which means that the surface of TiO_2 ceramics is covered with a protein molecule monolayer.

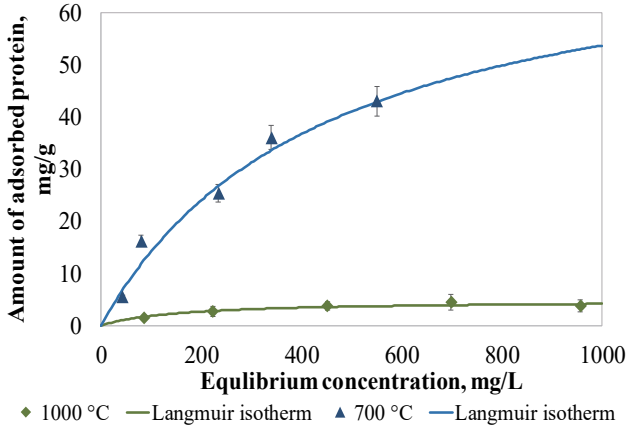


Fig. 8. Langmuir isotherms of protein sorption on thermally treated $nTiO_2$ powders.

***In vitro* bioactivity.** For a long time TiO_2 ceramics has been considered as bioinert, but recent studies have shown that in certain conditions it is possible to obtain *in vitro* bioactive TiO_2 ceramics that can induce apatite nucleation on its surface [11]. Pressed samples prepared from TiO_2 nanopowder were used to evaluate the impact of thermal treatment on *in vitro* bioactivity of obtained ceramics. Pressed pellets were sintered in two different thermal treatment regimes: one-step (conventional) and two-step sintering. Physical characteristics of the samples are shown in Table 1. Formation of apatite layer on the surface of thermally treated $nTiO_2$ is shown in Fig. 9. Results indicate that thermal treatment of ceramics significantly affects apatite precipitation. On the surface of the samples that were sintered at lower temperatures (600 °C, 700 °C, 800–700 °C), formation of apatite microspheres was observed after 5 days in SBF (Fig. 9). Increasing immersion time in SBF up to 21 days, the sample surface was completely covered with the apatite layer. The samples sintered at higher temperatures (770 °C, 800 °C and 900–700 °C) showed no *in vitro* bioactivity even after immersion in SBF for 28 days.

Table 1

Impact of Thermal Treatment on Rutile Phase Content and Microstructural Characteristics of $nTiO_2$ Ceramic Samples

Thermal treatment temperature, °C	Rutile phase content, %	Specific surface area, m^2/g	Grain size, nm
600	7.8 ± 0.4	66.1 ± 6.6	37 ± 4
700	12.1 ± 0.8	39.3 ± 3.9	46 ± 5
770	14.0 ± 0.7	33.0 ± 3.3	61 ± 6
800	25.0 ± 0.7	18.2 ± 1.8	103 ± 12
800–700	14.1 ± 1.0	30.1 ± 3.0	49 ± 6
900–700	35.3 ± 1.2	20.0 ± 2.0	90 ± 9

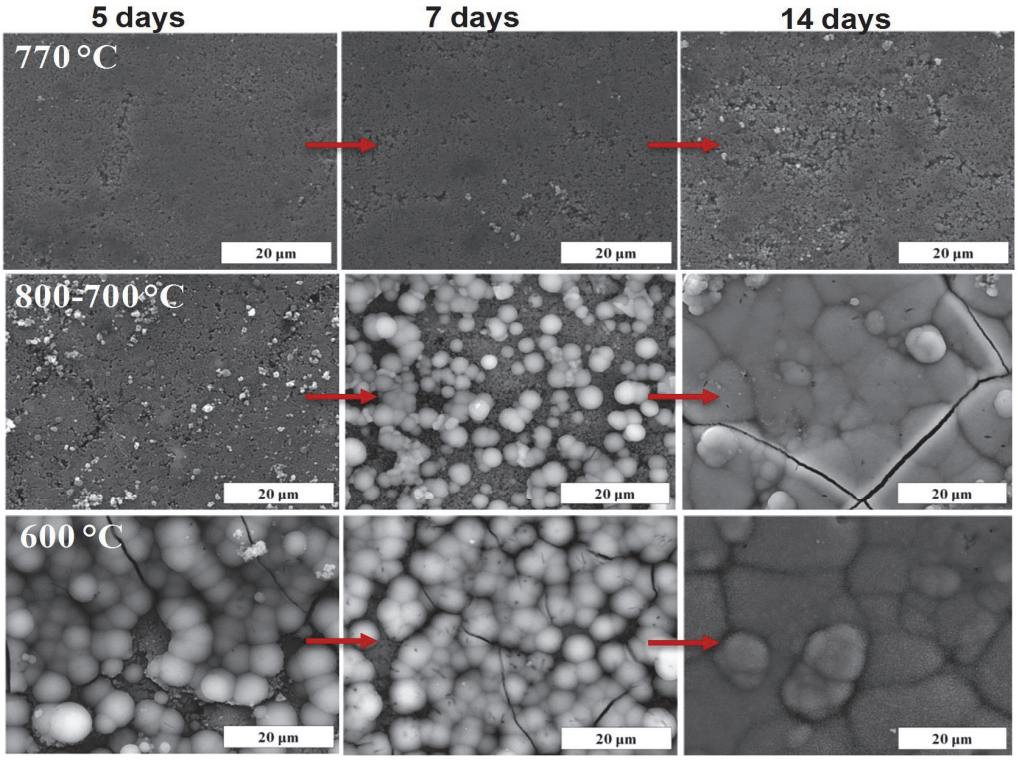


Fig. 9. SEM micrographs of the surfaces of the $nTiO_2$ ceramics after immersion in SBF.

Results demonstrate that bonelike apatite formation on $nTiO_2$ ceramics is affected by multiple factors, including grain size, specific surface area and phase composition. Results indicate that the essential factor for apatite formation is grain size. Higher specific surface area accelerates apatite formation due to the increased number of Ti-OH groups on the surface. The phase composition has minor influence on apatite-forming ability of $nTiO_2$ ceramics comparing to grain size and specific surface area.

Dynamics of apatite layer formation on *in vitro* bioactive sample surface was evaluated, *e.g.*, mass increment and thickness of formed layer depending on the immersion time in SBF.

Morphology and structure of the deposited layer were characterised using scanning electron microscopy, energy dispersive X-ray spectroscopy, X-ray diffraction (Fig. 10 (a)), Fourier transform infrared spectroscopy (Fig. 10

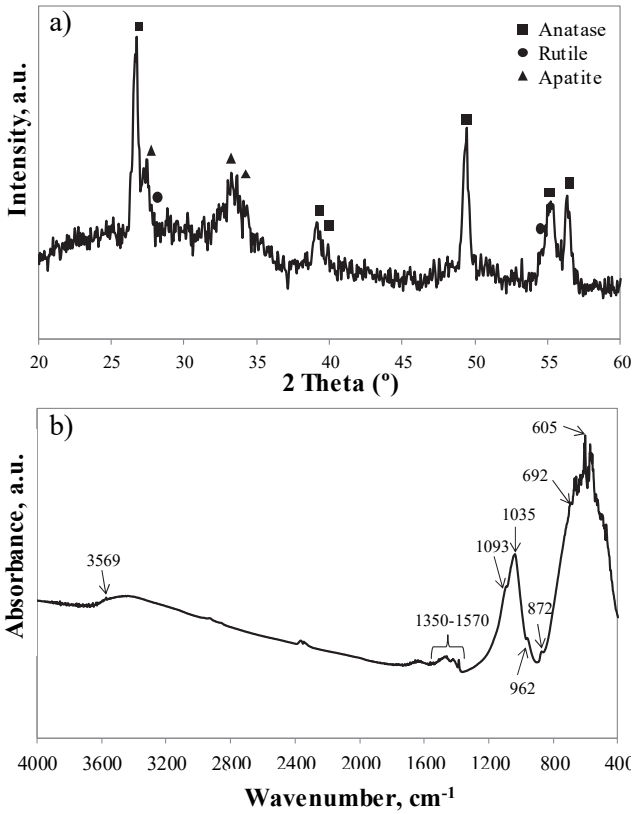


Fig. 10. (a) XRD pattern and (b) FT-IR spectrum of newly formed layer.

(b)) and *Raman* spectroscopy. The obtained results confirmed that the new layer formed on $nTiO_2$ surface was low crystallinity carbonate-containing hydroxyapatite or bonelike apatite that was similar to biological apatite in bones.

Bactericidal adhesion/colonisation. Implant-associated infections generally caused by microbial adhesion and followed by cell colonisation and bacterial biofilm formation on the artificial implants remain one of the most common causes of biomaterial implant failure in modern medicine. The bacterostatic effect of $nTiO_2$ ceramics sintered at 700 °C and 1000 °C in air, after subsequent vacuum heat treatment and irradiation with UV-light was investigated using *St.epidermidis* and *Ps.aeruginosa* bacteria colonies. Samples were cultivated for 2 hours at 37 °C in order to determine the intensity of adhesion and for 24 hours to determine the intensity of colonisation. The obtained results showed that with both microorganisms used in the study there was no adhesion or it was very inhibited on the surfaces of all samples. Comparing to other ceramic biomaterials, TiO_2 ceramics used in this study showed less ability to attract microorganisms, and colonisation intensity was low as well. Thus, all TiO_2 ceramic samples from bacteriostatic point of view can be used as a potential material in bone tissue repair.

2. Preparation and Characterisation of Porous TiO_2 Ceramic Scaffolds

Porous scaffolds were prepared via a polymer replica method. Impact of slurry technological parameters, thermal treatment and recoating on porosity, pore size and compressive strength of the obtained scaffolds was evaluated.

Slurry Production Technology

$mTiO_2$ anatase powder, 5 wt% polyvinyl alcohol solution (4 wt%) ethylene glycol (6 wt%) and deionised H_2O were used as raw materials to obtain ceramic slurry. Homogenization of ceramic slurry was performed by vigorous stirring at 1000 rpm and pH of the slurry was kept above 10 using 25 % ammonia solution. Thermal treatment was made at 1500 °C for 30 h. Effect of stirring time and TiO_2 content in the slurry on rheological properties was analysed.

Increasing stirring time, an average particle size decreases (Fig. 11 (a)). After 1 h of stirring, TiO_2 particles were deaglomarated and reached average size below 1 µm. Increasing stirring time up to 23 h, no significant particle size changes were observed, but viscosity and yield stress slightly increased that could be attributed to water evaporation and condensation on the walls of the container (Fig. 11 (b)). The optimum stirring time was chosen 3 h.

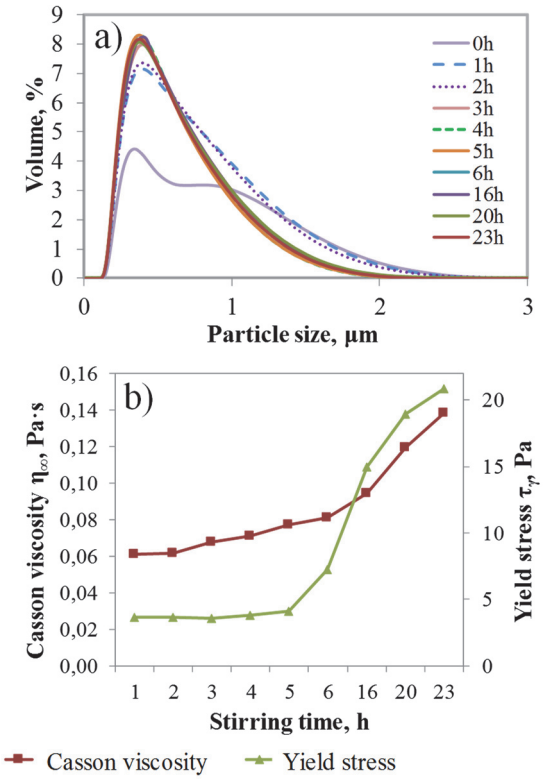


Fig. 11. (a) Particle size distribution of the slurry and viscosity and (b) yield stress as a function of stirring time.

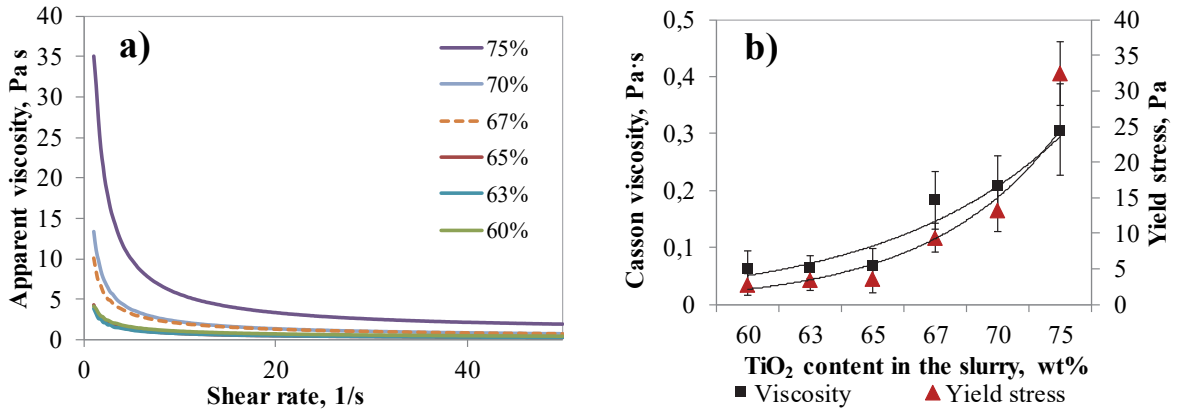


Fig. 12. (a) Apparent viscosity η of TiO_2 slurry as a function of shear rate and (b) *Casson* viscosity η_∞ depending on TiO_2 content in the slurry.

One of the most important parameters affecting rheological properties of the slurry and, therefore, the resulting properties of the scaffolds is the TiO_2 content in the slurry. As evident from Fig. 12 (a), apparent viscosity η decreases and shear rate γ increases. The observed phenomenon is characteristic of the pseudoplastic systems. Highly loaded suspensions, to which TiO_2 slurry can be attributed, can be regarded as structured disperse systems. With the increase in shear rate γ, the initial structure gradually collapses and, hence, viscosity decreases. Conversely, with the increase in the concentration of disperse phase, the degree of structural arrangement increases, which causes an increase in viscosity. Processing experimental data by the *Casson* model, *Casson* viscosity η_∞ and yield stress τ_γ of the slurry with different TiO_2 content were calculated. Increase of *Casson* viscosity η_∞ along with the increase of TiO_2 content in the slurry is shown in Fig. 12 (b). Increasing TiO_2 content from 60 wt% to 75 wt%, *Casson* viscosity increases more than 4 times.

Rheological properties of TiO_2 slurry significantly affect structure and properties of the obtained scaffolds. Macrostructure of TiO_2 scaffolds obtained from the slurry with different TiO_2 content is shown in Fig. 13. There are no major differences in the scaffold macrostructure changing TiO_2 content from 60 % to 70 %. As evident from Table 2,

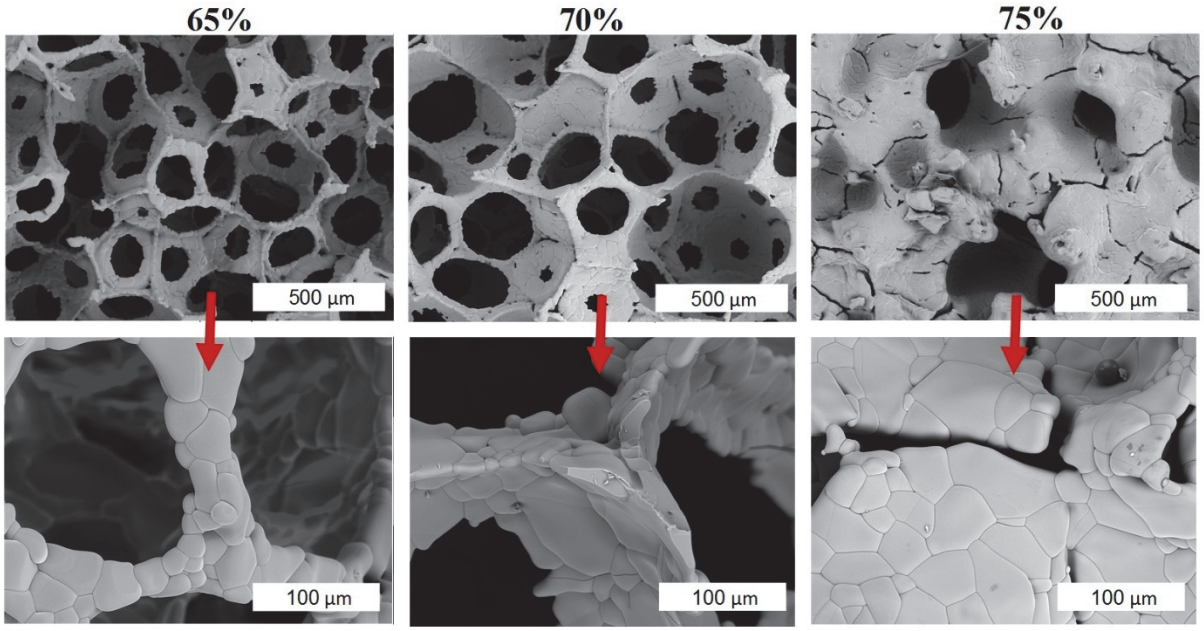


Fig. 13. Macrostructure of TiO_2 scaffolds depending on TiO_2 content in the slurry.

increasing TiO_2 content in the slurry, the ceramic wall thickness slightly increases, but porosity and pore size remain similar, i.e., above 94 % and from 50 μm to 400 μm.

Table 2

Impact of the TiO_2 Content in the Slurry on the Characteristics of TiO_2 Scaffolds

	Pore size, μm	Wall thickness, μm	Porosity, %	Compressive strength, MPa
60%	80 - 302	50 ± 33	97 ± 0.4	0.26 ± 0.07
63%	63 - 330	53 ± 19	97 ± 0.4	0.30 ± 0.05
65%	78 - 324	57 ± 27	96 ± 1	0.41 ± 0.15
67%	82 - 377	65 ± 31	94 ± 0.4	0.47 ± 0.10
70%	78 - 367	79 ± 42	94 ± 0.6	0.47 ± 0.11
75%	-	-	71 ± 8	1.78 ± 0.83

Scaffolds can be characterised with open and interconnected pore structure, which is essential for using them in bone tissue regeneration. The structure of PU precursor is not maintained by using slurry with TiO_2 content 75 wt%. Most of the pores are blocked and porosity reaches only (71 ± 8) %, but at the same time compressive strength significantly increases and reaches (1.78 ± 0.83) MPa that is 3.8 times higher than for scaffolds obtained from 70 wt% TiO_2 slurry. However, the use of such scaffolds in bone tissue engineering is limited because it will not allow ingrowth of the blood vessels and cell migration throughout the scaffold volume for which open porosity is desirable. Compressive strength of scaffolds obtained from slurry with TiO_2 content from 60 wt% to 70 wt% increases from (0.26 ± 0.07) MPa to (0.47 ± 0.11) MPa with increasing TiO_2 content.

Based on the rheological investigations, microstructure of obtained scaffolds and compressive strength values, for further studies slurry with TiO_2 content 65 wt% was chosen for scaffold production.

Impact of Thermal Treatment

One of the disadvantages of polymer replica method is the formation of voids in porous ceramic struts during polymer pyrolysis process. Therefore, it is necessary to optimise the heat treatment parameters in order to obtain mechanically resistant ceramics.

During thermal treatment, reduction of TiO_2 scaffold dimensions was observed. The thermal shrinkage of the scaffold volume reached ~30 % at 1100 °C, 50 % at 1200 °C and ~60 % at 1300 °C and higher temperatures.

Impact of thermal treatment on the microstructure of porous TiO_2 scaffolds is shown in Fig. 14. Scaffolds that are thermally treated at 1100 °C are at the initial stage of the sintering process – ceramic grains start to grow; cracks and triangular voids formed during PU foam pyrolysis in the ceramic struts are observed. These kinds of defects lower mechanical properties of ceramic material. Increasing the temperature, ceramic sintering process intensifies; voids and cracks in the ceramic struts disappear. Summarising the impact of sintering temperature and holding time at the evaluated temperature on porosity, pore size, wall thickness and compressive strength, it is concluded that porosity and pore size for all scaffolds are similar and in the range from 94 % to 97 % and 60 μm to 400 μm, respectively.

By increasing sintering temperature from 1200 °C to 1600 °C, compressive strength of the TiO_2 scaffolds increases from (0.05 ± 0.02) MPa to (0.42 ± 0.13) MPa or 8 times. These findings directly correlate to microstructural observations and confirm the fact that triangular voids lower mechanical properties.

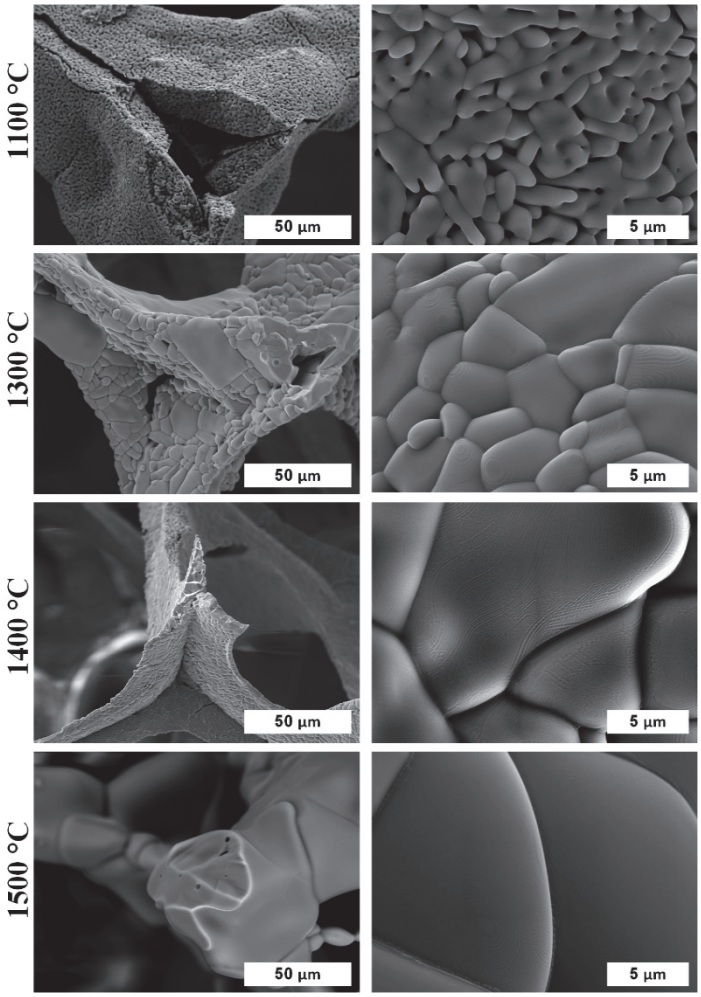


Fig. 14. Effect of thermal treatment temperature on microstructure of sintered TiO_2 scaffolds.

Increasing the holding time at the evaluated temperature (1500 °C) from 10 h to 30 h, compressive strength also increases from (0.24 ± 0.04) MPa to (0.41 ± 0.15) MPa. Cracking of ceramic grains was observed if the scaffolds were sintered for 40 h at 1500 °C.

By analysing the impact of heat treatment on the properties of TiO_2 scaffolds, it can be concluded that heat treatment should be realized at high temperatures. The optimal thermal treatment for production of porous TiO_2 scaffolds is 1500 °C with a holding time of 30 h or 1600 °C with the holding time of 10 h.

Recoating of TiO_2 Scaffolds with $mTiO_2$ Suspension

To increase the mechanical strength, porous scaffolds were recoated with low viscosity suspension ($mTiO_2$ amount in suspension – 30 wt%) using a vacuum impregnation method. After every recoating and drying scaffolds were thermally treated at 1500 °C for 30 h. During sintering no additional shrinkage of TiO_2 scaffolds was observed. As shown in the SEM micrographs, ceramic walls of porous scaffolds were covered with a thin layer of $mTiO_2$ coating, remaining open and interconnected pore structure (Fig. 15).

By recoating scaffolds from 1 to 4 times, porosity and pore size do not change significantly, but slightly increase the average strut size or wall thickness from (47 ± 13) µm to (73 ± 33) µm (Table 3). By recoating the scaffolds, the voids and cracks in the ceramic walls are filled with TiO_2 particles and, as a result, their mechanical properties increase. Recoating scaffolds four times and thermally treating at 1500 °C for 30 h, the compressive strength at the porosity of 92 % reaches (1.75 ± 0.45) MPa that is comparable to the compressive strength of trabecular bone.

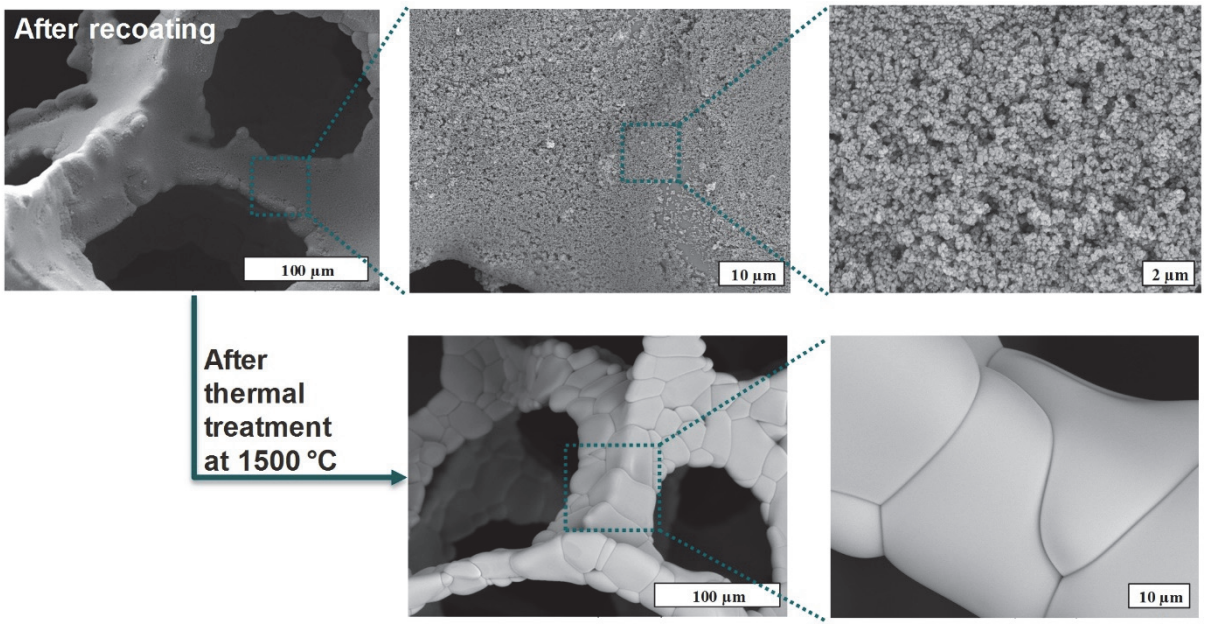


Fig. 15. Microstructure of TiO_2 scaffolds after recoating with $mTiO_2$ suspension.

Table 3

Impact of the Applied Coatings on the Characteristics of TiO_2 Scaffolds

Applied coatings	Wall thickness, µm	Compressive strength, MPa
1st coating	47 ± 13	0.62 ± 0.17
2nd coating	56 ± 25	1.25 ± 0.19
3rd coating	66 ± 36	1.47 ± 0.33
4th coating	73 ± 33	1.75 ± 0.45

Electrically Active TiO_{2-x} Scaffolds

Bone healing can be significantly improved by applying electrical stimuli in the injured region [12]. Thus, electrically active scaffolds with 3D structure are of interest as bone graft substitute materials. Such materials can locally deliver electrical current to the cells in the bone defects and at the same time ensure space for new bone formation. Preparation of novel highly porous and electrically active TiO_{2-x} ceramic scaffolds via a polymer replica method was proposed. Scaffolds showed fully open and interconnected pore structure with porosity of around 95 %. Thermal treatment of the

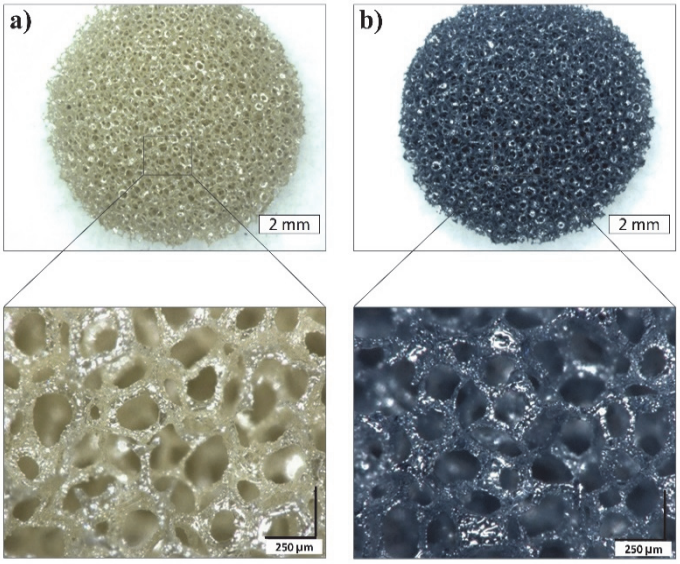


Fig. 16. Digital microscopy images of (a) TiO_2 scaffold and (b) electrically active TiO_{2-x} scaffold.

scaffolds under high vacuum conditions was realized to obtain nonstoichiometric TiO_{2-x} scaffolds. Electrical conductivity of the TiO_2 scaffolds after sintering in air was relatively low (10^{-8} mS/m to 10^{-9} mS/m). Thermal treatment of the scaffolds under high vacuum conditions caused a dramatic increase in electrical conductivity (44 mS/m to 90 mS/m) that was comparable to semiconductors.

During TiO_2 scaffold sintering under high vacuum conditions colour changed from yellow to dark blue/grey (Fig. 16). Blue colouration of the TiO_{2-x} scaffolds can be explained by creation of colour centres. Colour centres are associated with bulk defects that are formed upon reduction of TiO_2 and significantly improve electrical conductivity of the material. *In vitro* studies confirmed that scaffolds were cytocompatible and enhanced cell spreading. Thus, TiO_{2-x} scaffolds hold a potential to be used in bone tissue regeneration as an electrical stimuli supplier enhancing the bone healing process.

3. Coatings on Porous TiO_2 Scaffolds

Different methods like coating with inorganic compounds, polymers or their composite materials are used to improve scaffold properties (mechanical properties, surface properties, bioactivity, antibacterial properties, degradation, *etc*.). In the experimental work, porous TiO_2 scaffolds were modified by coating them with nanosized TiO_2, polymers (Ch, PLA, PVA) or HAp/PVA composites to improve compressive strength and *in vitro* bioactivity.

Nanosized TiO_2 Coating on TiO_2 Scaffolds

Previous research showed that *in vitro* bioactivity of TiO_2 ceramics depended on thermal treatment conditions. In order to obtain mechanically resistant porous scaffolds, they should be thermally treated at high temperatures (1500 °C). TiO_2 ceramics sintered at high temperatures does no induce apatite formation on its surface. It implies that modifying mechanically resistant porous TiO_2 scaffolds with nanosized TiO_2, their bioactivity should be improved.

TiO_2 scaffolds were coated with $nTiO_2$ using a vacuum impregnation method or electrophoretic deposition (EPD) and thermally treated at 700 °C. Although a vacuum infiltration method is quite simple, it is difficult to repeat and coating thickness can vary. Thus, EPD was selected as a method to obtain uniform coating and by changing EPD parameters (time and voltage) it was easy to adjust thickness and morphology of the coating [13]. It is possible to obtain electrically conductive TiO_{2-x} ceramic that can be used as a substrate/electrode in EPD. The most important factors that influence EPD is suspension stability, deposition time and applied voltage [13].

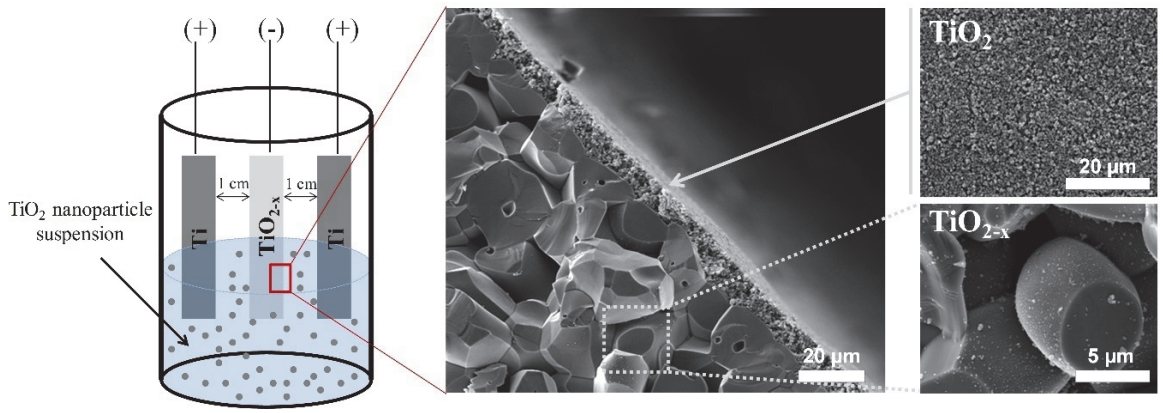


Fig. 17. Schematic representation of electrophoretic deposition on dense TiO_{2-x} ceramics.

Significant factor that affects the quality of the coating is stability of the suspension. The suspensions were prepared by adding 0.2 g of TiO_2 nanopowder to 100 ml isopropanol, which contained different concentrations of triethanolamine (TEA) as a dispersant, and magnetically stirred for 1 h followed by ultrasonification for 5 min. TEA concentration varied from 0 ml/L to 40 ml/L. The sedimentation test was used to determine the optimal concentration of TEA in order to obtain the most stable suspensions.

Suspensions without TEA addition (0 ml/l) are unstable and rapid settling of the $nTiO_2$ particles is observed. TEA addition increases stability of the suspensions and optimum concentration of TEA was selected to be 10 mL/L that was used throughout the further EPD experiments. Analysis of morphology revealed that some TiO_2 nanoparticle agglomerates were evident in 10 ml/L suspension.

In order to determine the optimal EPD parameters dense TiO_{2-x} ceramics was used. Schematic representation of electrophoretic deposition on dense electrodes is shown in Fig. 17. TiO_{2-x} ceramic and titanium plates were used as electrodes for direct current EPD. The distance between the substrate and counter electrodes was set to 1 cm. EPD experiments were carried out varying deposition time and voltage. Deposition yield was calculated from deposition mass and covered surface area. After EPD, samples were thermally treated in the temperature range from 700 °C to 1100 °C in order to evaluate the impact of sintering on phase composition and microstructure of obtained coatings.

An increase in the deposition time at the constant voltage ($V = 20$ V) linearly increases the deposition yield and calculated thickness of the coating as illustrated in Fig. 18 (a). Increasing the applied voltage at the constant time (20 min), deposition yield and calculated thickness also increase linearly at relatively low voltage values (below 40 V) (see Fig. 18 (b)). The reduction in the deposition rate was observed by increasing the deposition voltage above 40 V. It can be explained by the formation of insulator layer of TiO_2 nanoparticles on the electrode surface that reduces current density and, as a result, inhibits nanoparticle deposition.

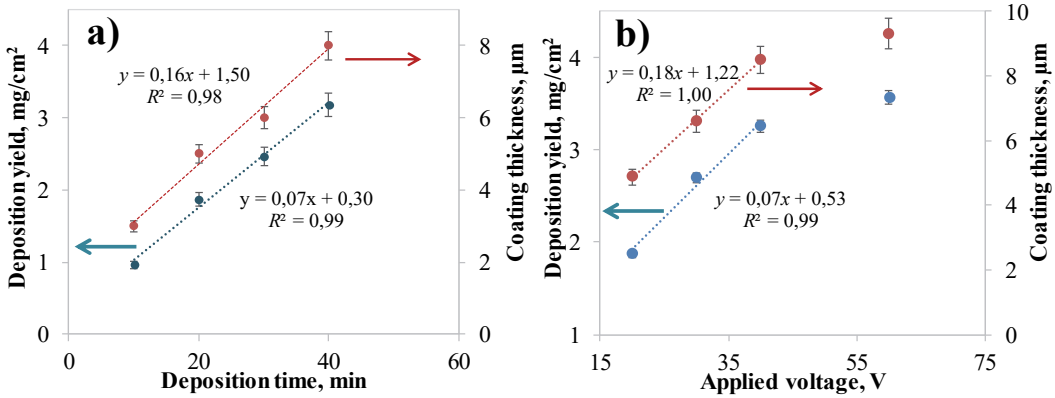


Fig. 18. Deposition yield and calculated thickness of the $nTiO_2$ coatings against (a) deposition time ($V = 20$ V) and (b) deposition voltage ($t = 20$ min) on dense TiO_{2-x} electrodes.

Thermal treatment from 700 ºC to 1100 ºC for 1 h was used to improve adhesion of the coating to the substrate. It causes anatase to rutile phase transformation (Fig. 19). The samples annealed at 700 °C exhibit mainly the anatase phase. Complete phase transformation from anatase to rutile crystalline modification occurs at 900 °C.

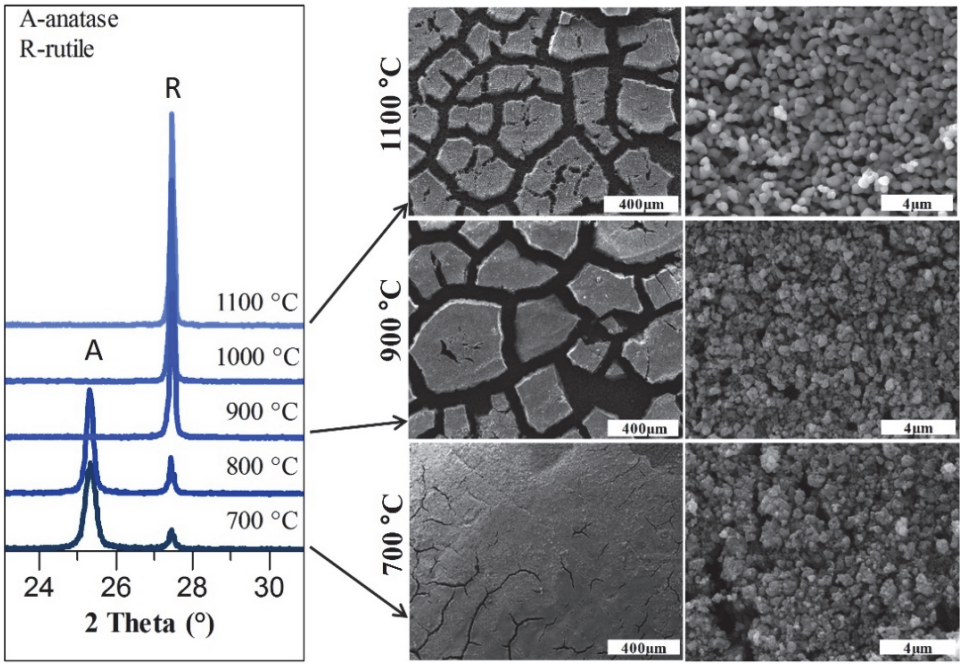


Fig. 19. Impact of thermal treatment on phase composition and surface microstructure of $nTiO_2$ coating.

Increasing sintering temperature to 1100 °C agglomerated particles grow together and develop in ceramic grains with average size of 400 nm. Crack formation during sintering was observed that could be attributed to thermal stresses and phase transformation. The results obtained indicate that the heat treatment should be carried at 700 °C, thus ensuring TEA decomposition, anatase phase and nanostructured grains needed to obtain bioactive coating.

Realizing electrophoretic deposition on porous TiO_{2-x} electrodes, open and interconnected pore structure of the scaffolds was maintained. Nanoparticles were deposited only on the surface of ceramic walls. As shown in Fig. 20, deposition yield increases linearly by increasing deposition time or applied voltage, as it was observed in the case of dense electrodes. If the electrophoretic deposition time is too short (below 10 min), the surface of ceramic walls is not completely covered with TiO_2 nanoparticles. Increasing deposition time, a relatively uniform coating can be obtained. Also by using a too low voltage (below 10 V), ceramic walls are not completely covered. If the voltage is to high (30 V), $nTiO_2$ particles deposit on the nearest surface that on their migration way form a thick layer on the one side of the wall and the other side leave uncoated (Fig. 21). The best quality of $nTiO_2$ coating on porous TiO_{2-x} scaffold was obtained at 20 V for 20 min.

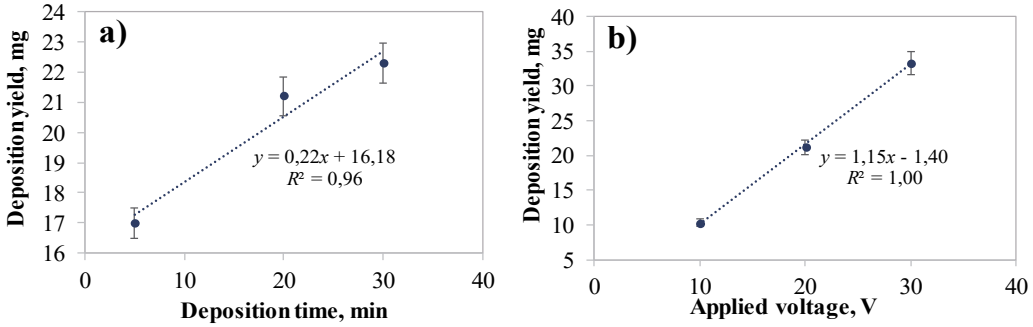


Fig. 20. Deposition yield of the $nTiO_2$ coatings against (a) deposition time (V = 20 V) and (b) deposition voltage (t = 20 min) on porous TiO_{2-x} electrodes.

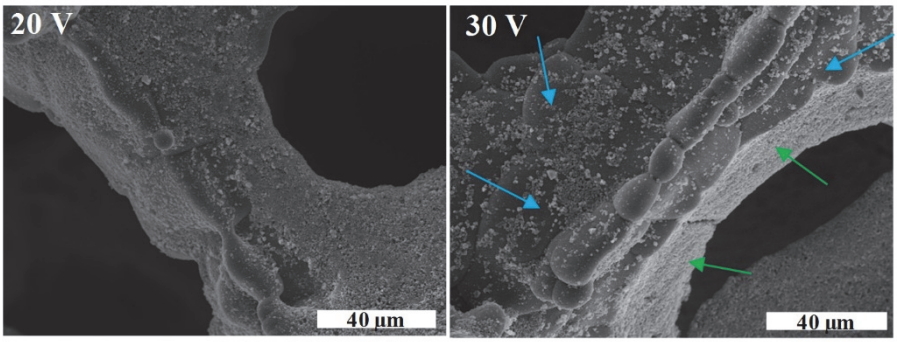


Fig. 21. SEM micrographs of $nTiO_2$ coating on porous TiO_2 electrodes obtained by electrophoretic deposition varying deposition voltage at t = 20 min.

Assessment of *in vitro* bioactivity of $nTiO_2$ coated scaffolds was performed by immersing in simulated body fluid up to 21 days. As evident from SEM micrographs (Fig. 22 (a)), formation of sphere-like particles on the surface of $nTiO_2$ coated scaffolds was observed. By increasing the immersion time in SBF, the ceramic walls were uniformly covered with newly formed particles. After scaffold immersion in SBF, new broad diffraction peaks centred at 2θ around 33° were detected in XRD pattern. These peaks can be attributed to the low crystalline or nanocrystalline apatite. FT-IR spectra of coated scaffolds before and after immersion in SBF are shown in Fig. 22 (b). Absorption bands attributed to PO_4^{3-} and CO_3^{2-} were detected, which indicated that deposits were B type CO_3^{2-} substituted apatite. Obtained results confirmed the formation of apatite microspheres on the surface of $nTiO_2$ coated scaffolds. Thus, bioactive and 3D highly porous TiO_2 scaffolds were developed.

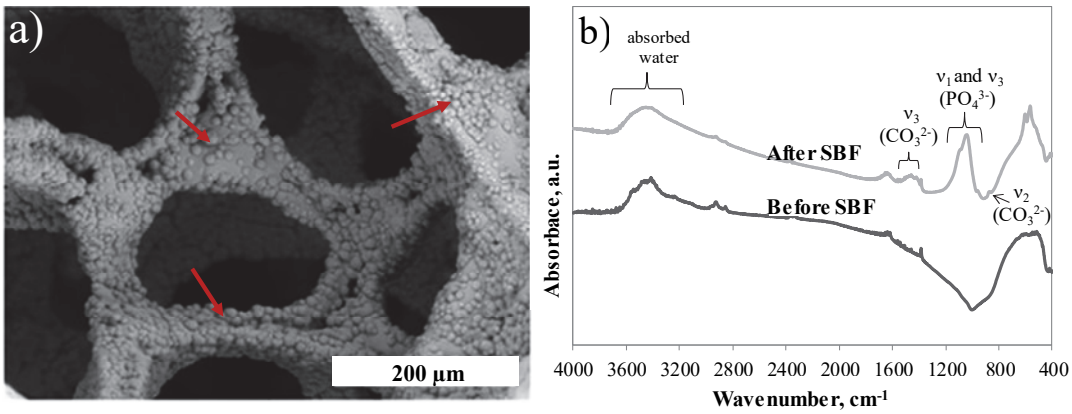


Fig. 22. (a) SEM micrograph and (b) FT-IR spectra of $nTiO_2$ coated scaffolds after immersion in SBF.

Polymer Coating on Porous TiO_2 Scaffolds

TiO_2 ceramic scaffolds were coated with different polymers to improve mechanical properties. Chitosan was selected as a natural biodegradable polymer with antibacterial properties, and PVA and PLA – as synthetic, biocompatible polymers. Polymer solutions were prepared by dissolving polymer in solvent (5 wt% PLA in dichloromethane, 2.5 wt% Ch in 1 % acetic acid and 5 wt% PVA in water) and further TiO_2 scaffolds were coated using a vacuum impregnation method followed by blowing with compressed air. By coating TiO_2 ceramic scaffolds with polymer, a few micron thick layer can be obtained (Fig. 23). Coated scaffolds remained the open and interconnected pore structure of TiO_2 scaffolds. Nevertheless, some pores were covered

with a polymer film and, as a result, porosity slightly decreased from 94 % to 88 %, but it was still high enough to use scaffolds in bone tissue engineering.

Compressive strength of PLA and Ch coated TiO_2 scaffolds significantly increased, in the case of Ch coated scaffolds even by 50 % (Fig. 24).

In vitro degradation of polymer coated scaffolds was assessed. Ch and PLA coated TiO_2 scaffolds did not show significant mass or microstructural changes, e.g., they still were coated with a polymer layer after removal from Tris-HCl buffer solution after 28 days. Compressive strength slightly decreased after *in vitro* degradation test, but still was higher than for uncoated TiO_2 scaffolds (Fig. 24).

In vitro bioactivity test revealed that Ch or PLA coated TiO_2 scaffolds did not promote biomimetic apatite formation on their surface even after immersion in SBF for 28 days.

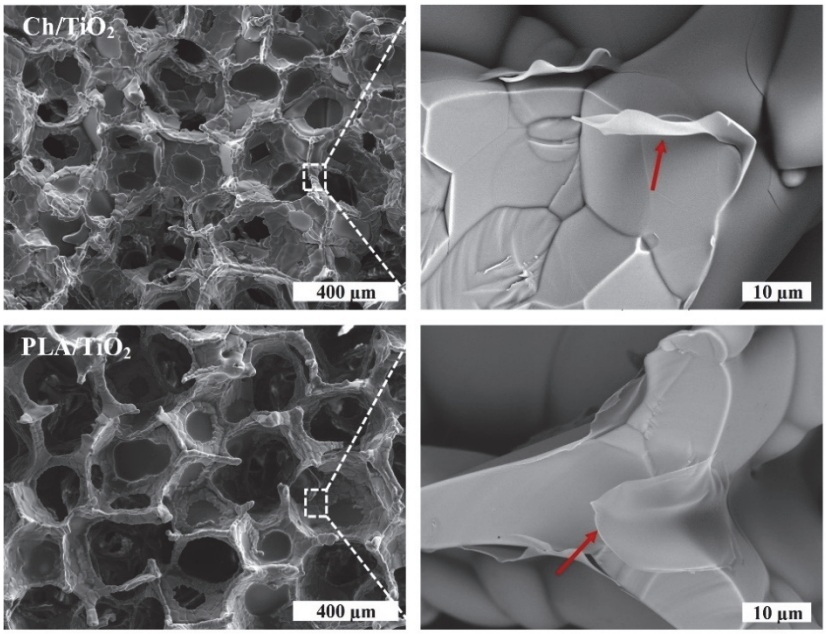


Fig. 23. Ch and PLA coating on porous TiO_2 ceramic scaffolds.

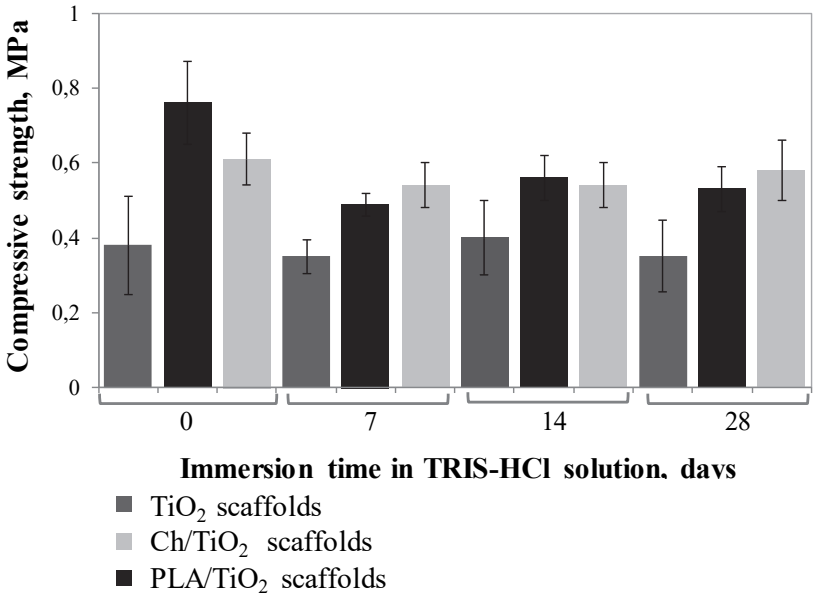


Fig. 24. Compressive strength of Ch or PLA coated TiO_2 scaffolds after *in vitro* degradation test.

Hydroxyapatite/Poly(vinyl alcohol) Coating on Porous TiO_2 Scaffolds

Development of novel tissue engineering scaffolds with suitable initial mechanical properties and favourable microstructure based on biodegradable polymer (PVA)/inorganic nanocomposite (HAp) and porous TiO_2 ceramics have been described. HAp were synthesized with a Ca/P molar ratio of 1.67 in the presence of 20 wt% aqueous solution of PVA at concentration of 30 wt% (HAp/PVA ratio) by reacting $Ca(OH)_2$ and H_3PO_4 at temperature of 45 °C. Obtained precipitates were aged at ambient temperature for ~20 h. Results showed that synthesising HAp *in situ* in the presence PVA, the formation of chemical bonding between HAp and PVA occured, such as

hydrogen bonding and/or hydroxyl-calcium-hydroxyl ([HO–]–Ca^{2+}–[–OH]) bonding, allowing for the uniform dispersion of HAp in the PVA matrix.

TiO_2 scaffolds were coated through a vacuum-assisted impregnation method followed by blowing with compressed air to remove an excess solution. As evident from SEM micrographs (Fig. 25), the original macroporosity and open pore structure of the TiO_2 scaffolds were maintained and a uniform coating from 1 μm to 4 μm was formed. Slightly decreased porosity of HAp/PVA coated scaffolds reached the value of (81 ± 2) %. Average pore size of the scaffolds was in the range from 100 μm to 500 μm. In addition, the crack-like defects as well as micropores and the struts were filled with PVA or HAp/PVA nanocomposite. This had a great impact on the mechanical strength. While the compressive strength of the TiO_2 scaffolds was (0.53 ± 0.16) MPa, the bioceramic composite consisting of PVA ((0.64 ± 0.21) MPa) or HAp/PVA ((0.99 ± 0.19) MPa) coatings could take higher loads in compression.

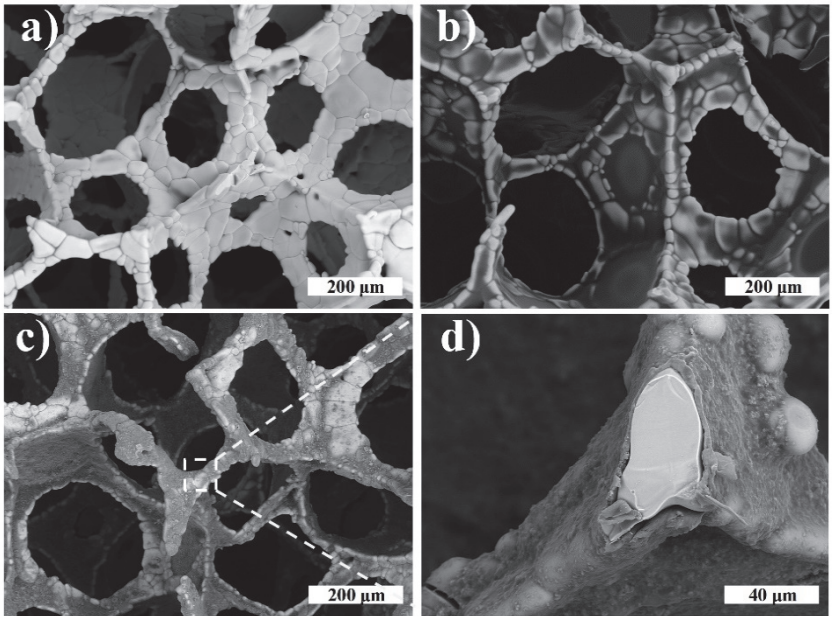


Fig. 25. FE-SEM micrographs of (a) TiO_2 scaffold; (b) PVA coated TiO_2 scaffold, (c) HAp/PVA coated TiO_2 scaffold and (d) cross-sectional view of HAp/PVA coated TiO_2 scaffold.

In vitro degradation test showed partial degradation of composite material. HAp/PVA coated scaffolds released fixed amount of Ca^{2+} ions (10 ppm) in Tris-HCl solution over the period of 1 to 28 days that probably arose mainly from the collapse of the chemical bonds between HAp and PVA. Thus, the Ca^{2+} ions bound to the PVA matrix were released first. However, the growing tendency of the weight loss indicated a gradual degradation of the HAp/PVA nanocomposite coating compared to rapid degradation of the PVA coating that dissolved within 5 hours. After immersion of the HAp/PVA coated TiO_2 scaffolds in Tris-HCl solution, the compressive strength of the coated scaffolds significantly decreased and reached initial values of TiO_2 scaffolds

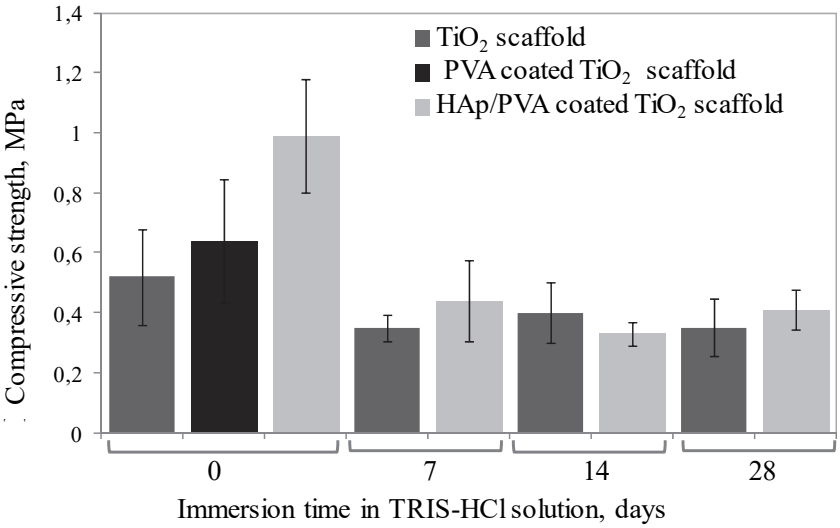


Fig. 26. Compressive strength of TiO_2 and HAp/PVA coated TiO_2 scaffolds after *in vitro* degradation studies.

(Fig. 26). This allows concluding that the improvement of the initial mechanical properties of the porous TiO_2 scaffolds is mainly due to the presence of PVA.

In vitro bioactivity assessment was carried out by immersion of the scaffolds in SBF for 7 days (Fig. 27). Formation of white sphere-like particles on the surface of HAp/PVA coated scaffolds was observed (Fig. 26). The molecular structure of newly formed particles was examined by FT-IR spectroscopy (Fig. 28). A significant increase in the intensity of the absorbance bands attributable to the phosphate groups [PO_4] of HAp phase indicated the apatite-like origin of the newly formed particles. Thus, the *in vitro* bioactivity testing proved high bioactive properties of the HAp/PVA nanocomposite coated TiO_2 scaffolds.

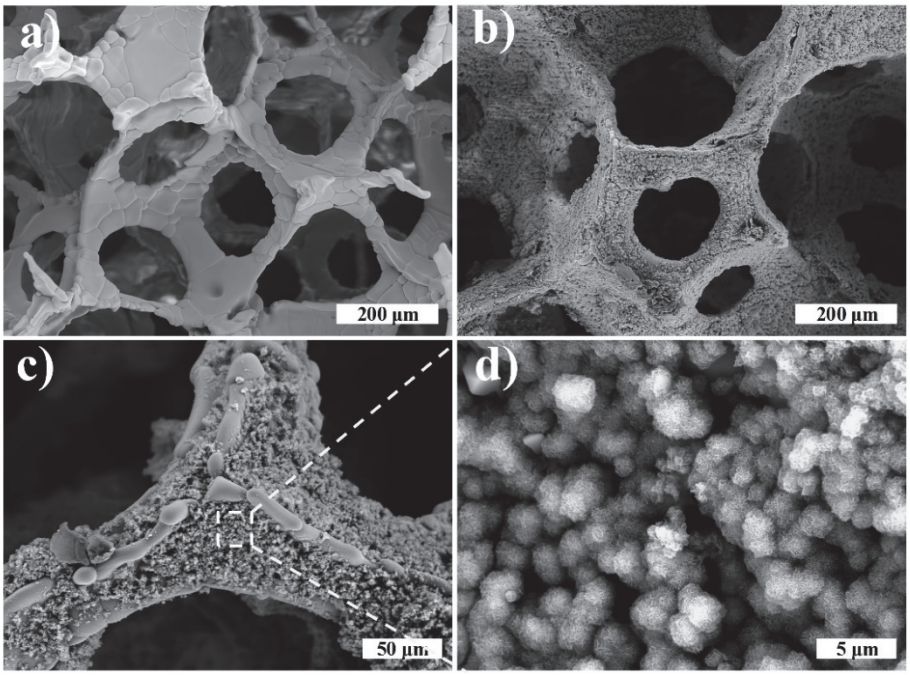
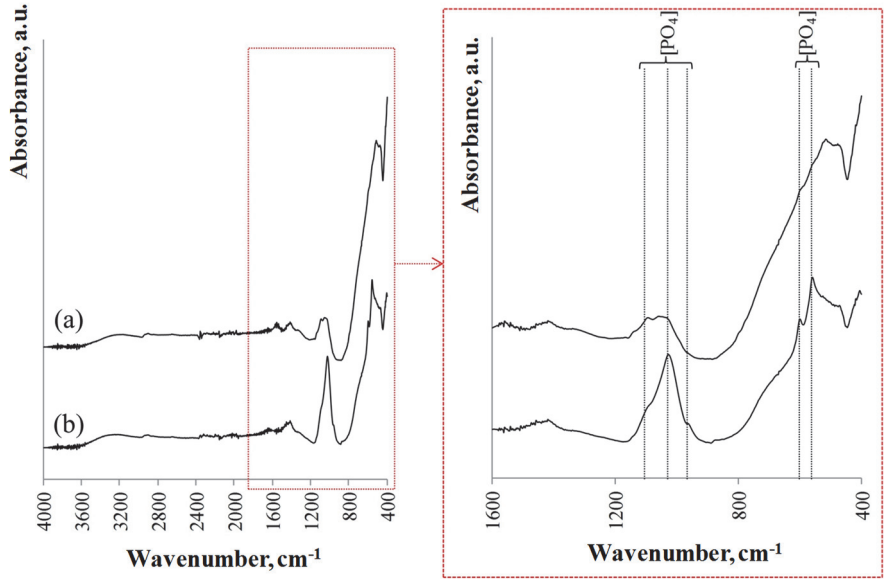


Fig. 27. FE-SEM micrographs of (a) TiO_2 scaffold and HAp/PVA coated TiO_2 scaffold after immersion in SBF for 7 days with different magnifications (b) 350 x, (c) 1 kx and (d) 10 kx.



28. att. FT-IR spectra of HAp/PVA coated TiO_2 scaffold (a) before and (b) after immersion in SBF for 7 days.

4. *In Vitro* Cell Studies

Assessment of cytocompatibility is very important for biomaterials used in bone tissue regeneration because it shows cell and material interactions that directly affect new bone formation. Influence of the obtained scaffolds on dermal fibroblast and osteoblast MG63-GFP adhesion and proliferation on the surface of the material was evaluated.

The interaction of 3 different types of porous scaffolds (thermally treated in air at 1500 °C for 30 h; after additional heat treatment under high vacuum condition; coated with nanosized TiO_2) with human dermal fibroblast cells after 10 days of cultivation was similar – the cells attached to all ceramic scaffolds at different levels (Fig. 28). Fibroblasts showed their typical morphology – spindle-shaped structure (Fig. 28).

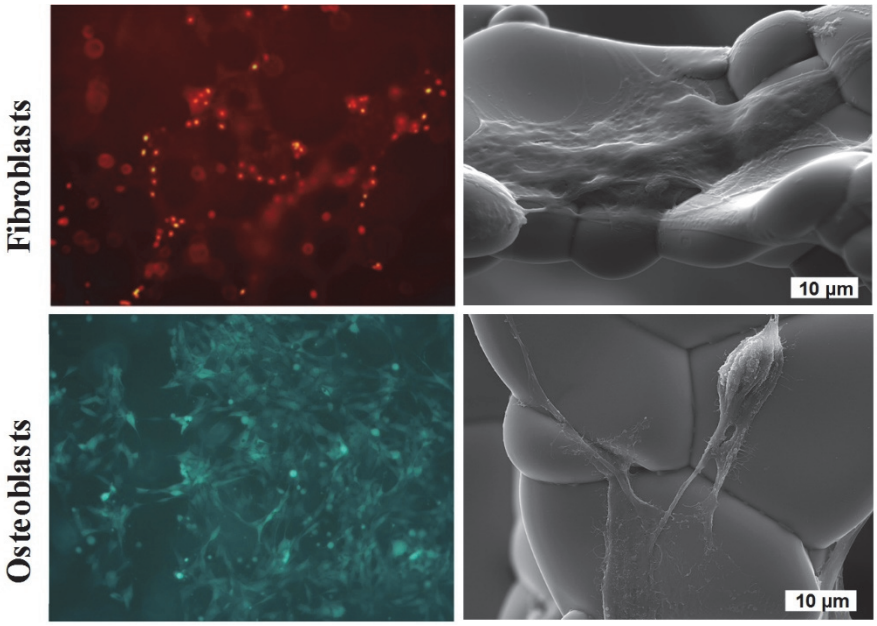


Fig. 28. Fluorescent microscopy and SEM images of osteoblast and fibroblast adhesion on TiO_2 scaffolds.

Similarly, osteoblast MG63-GFP cells attached to the scaffold surface at different depths and showed a spindle-shaped structure (Fig. 28). In addition, some cells were forming clusters in-between the pores. All three types of scaffolds showed a similar osteoblast MG63-GFP proliferation rate that was slightly lower compared to control (Fig. 29). Cultivating cells on the scaffolds for 3 days, the number of the cells increased two times. This indicated that scaffolds presented a favourable microenvironment for cell attachment and growth. The spreading cells maintained physical contact with each other through filopodia or lamellopodia. Results indicated that scaffolds were not cytotoxic and were biocompatible.

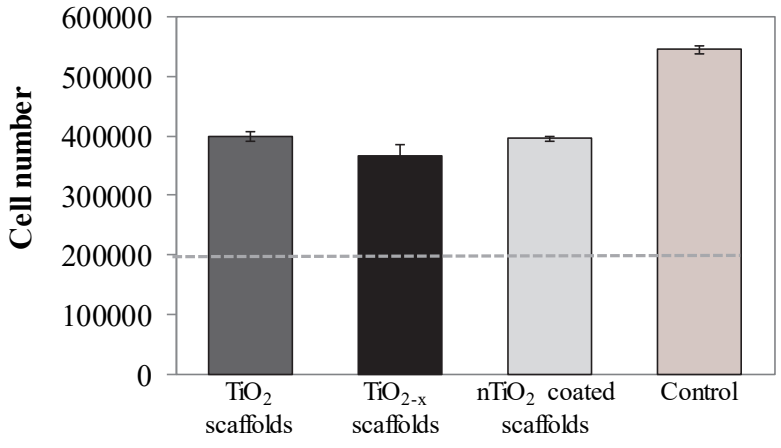


Fig. 29. Proliferation rate of osteoblasts MG63-GFP on the scaffold surface after 3 days in culture medium.

Apparently, modification of porous TiO_2 ceramic scaffolds (conductive TiO_{2-x} ceramics and coated with nano-sized TiO_2 particles) was not essential to cell attachment and growth.

CONCLUSIONS

1. Particle size of TiO_2 raw material affects phase transformation, sintering process, density, grain size and surface properties of the obtained ceramics. Decreasing particle size phase transformation occurs at lower temperatures (for TiO_2 powder with particle size of 180 nm in the temperature range from 900 °C to 1100 °C and 15 nm – 600 °C to 900 °C) and, as a result, denser ceramics with smaller grain size and higher surface energy can be obtained.
2. Formation of bonelike apatite on the surface of TiO_2 ceramics depends on thermal treatment that affects a surface microstructure. Ceramic scaffolds with nanosized grains (<50 nm) and higher surface area (>30 m^2/g) induce faster apatite formation. Ceramic scaffolds do not promote bactericidal adhesion and colonisation of *Stafilococus epidermidis* and *Pseudomonas aeruginosa* on a ceramic surface.
3. Slurry content and production technology affect physico-mechanical properties of highly porous TiO_2 ceramic scaffolds obtained via a PU replica method. Optimal slurry production parameters were determined in order to obtain 3D highly porous TiO_2 ceramic scaffolds with an open and interconnected pore structure, porosity above 90 % and compressive strength up to 1.8 MPa.
4. Compressive strength of TiO_2 scaffolds can be increased by 2 to 3 times by coating with low concentration TiO_2 slurry (30 wt%) or biocompatible polymers (Ch, PLA, PVA) and at the same time remaining a highly porous structure.
5. Thermal treatment of TiO_2 ceramics causes formation of nonstoiciometric TiO_{2-x} ceramics that further affects electrical properties. Electrically active and 3D porous TiO_{2-x} ceramic scaffolds have been obtained for the first time. Electrical conductivity of porous TiO_{2-x} ceramic scaffolds is >40 mS/m that is comparable to semiconductors. Electrically active scaffolds can be used to apply electrical stimuli in bone defects and enhance bone healing.
6. Homogenous nanosized TiO_2 coating on porous, electrically active TiO_{2-x} scaffolds have been obtained for the first time via direct current electrophoretic deposition. TiO_2 scaffolds coated with TiO_2 nanoparticles show *in vitro* bioactivity.
7. Compressive strength from (0.53 ± 0.16) MPa to (0.99 ± 0.19) MPa and *in vitro* bioactivity of TiO_2 scaffolds can be improved by coating them with PVA/HAp composite. HAp particles act as active centres for apatite nucleation on TiO_2 scaffolds. Due to the fast degradation of PVA, such composite material can be used as a local drug delivery system.
8. *In vitro* cell studies have confirmed that the obtained scaffolds are cytocompatible due to good adhesion and normal growth of dermal fibroblast and osteoblast MG63-GFP cells on a scaffold surface.

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APPROBATION OF THE RESULTS

The scientific results of the Doctoral Thesis have been summarised in 12 full text scientific articles (10 of them indexed in SCOPUS database) and presented at 21 international conferences.

List of Publications

1. I.Narkevica, L.Stipniece, E.Jakobsons A.Caksa-Lapsina, and J.Ozolins. **Electrically active and 3D porous TiO_{2-x} ceramic scaffolds for bone tissue regeneration**. *Journal of the European Ceramic Society,* 2017, 37, pp. 833–840, DOI 10.1016/j.jeurceramsoc.2016.09.032 (SCOPUS).
2. I.Narkevica, L.Stradina, L.Stipniece, and J.Ozolins. **Electrophoretic deposition of TiO_2 nanoparticles on dense TiO_{2-x} ceramic electrodes**. *Key Engineering Materials* 2016, 721, pp. 177–181, DOI 10.4028/www.scientific.net/KEM.721.177 (SCOPUS).
3. L.Stipniece, K.Salma-Ancane, V.Rjabovs, I.Juhnevica, M.Turks, I.Narkevica, and L.Berzina-Cimdina. **Development of functionalized hydroxyapatite/poly(vinyl alcohol) composites.** *Journal of Crystal Growth*, 2016, 44, pp. 14–20, ISSN 0022-0248, DOI 10.1016/j.jcrysgro.2016.03.029 (SCOPUS).
4. L.Stipniece, I.Narkevica, M.Sokolova, J.Locs, and J.Ozolins. **Novel scaffolds based on hydroxyapatite/poly(vinyl alcohol) nanocomposite coated porous TiO_2 ceramics for bone tissue engineering**. *Cermics International*, 2016, 42, pp. 1530–1537, ISSN 0272-8842, DOI 10.1016/j.ceramint.2015.09.101 (SCOPUS).
5. D.Loca, I.Narkevica, J.Ozolins, and L.Berzina-Cimdina. **The effect of TiO_2 nanopowder coating on *in vitro* bioactivity of porous TiO_2 scaffolds**. *Materials Letters*, 2015, 159, pp. 309–312, ISSN 0167-577X, DOI 10.1016/j.matlet.2015.07.017 (SCOPUS).
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7. I.Narkevica, L.Stradina, V.Yakushin, and J.Ozolins. **Preparation and characterization of porous titania ceramic scaffolds.** *RTU Material Science and Applied Chemistry*, 2015/31, pp. 5–9, ISSN 2255-8713, DOI 10.7250/msac.2015.001.
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10. I.Narkevica, M.Reimanis, J.Ozoliņš, J.Kleperis, L.Bērziņa-Cimdiņa. **Electrochemical studies of nonstoichiometric TiO_{2-x} ceramic.** *Key Engineering Materials*, 2014, 604, pp. 254–257, ISSN 1662-9795, DOI 10.4028/www.scientific.net/KEM.604.254 (SCOPUS).

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Participation in Scientific Conferences

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2. I.Narkevica, J.Ozolins, I.Cakstina, and I.Skadins. Fabrication and characterization of modified titanium dioxide scaffolds for bone tissue engineering. *9th annual meeting of the Scandinavian Society for Biomaterials*. Reykjavik, Iceland, 1–3 June 2016.
3. I.Narkevica, L.Bugovecka, L.Stradina, J.Ozolins, and L.Berzina-Cimdina. Porous titanium dioxide ceramic scaffolds for bone tissue regeneration. *LU Cietvielu fizikas institūta 32. zinātniskā konference*, Riga, Latvia, 17–19 March 2016.
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