RIGA TECHNICAL UNIVERSITY

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IMPACT OF PORE FORMING AGENTS ON STRUCTURE AND QUALITIES OF BIOCERAMIC MATERIALS

Summary of the Doctoral Thesis

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RIGA TECHNICAL UNIVERSITY
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Summary of the Doctoral Thesis

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CONFIRMATION

I confirm that I have elaborated Doctoral Thesis which I submitted for consideration at the Riga Technical University for acquisition of a Doctoral degree in engineering sciences. The present Doctoral Thesis is not submitted in other scientific institutions for acquisition of a scientific degree.

Rita Seržāne …… (Signature)

Date: ……

The Doctoral Thesis is written in Latvian, it contains introduction, literature review (6 chapters), experimental part (4 chapters), results and analysis (5 chapters), conclusions and the list of used references. The Doctoral Thesis contains 118 pages, 61 figures, 14 tables, 10 equations, 110 references and attachment.

GENERAL DESCRIPTION

State of the Art

The main feature of the new millennium is improving quality of human life. Development of biomaterials and their use for implant production, substitution of damaged tissues is related to changes in quality of life as well. The reason for the need of „body spare parts" is the body tissues damaged over the years. Particularly bone tissues are subject to age-related changes. Women are more sensitive - at the age of 35 - 60 years woman's bone density decreases for about 40% (about 20% for men). Bone density decreases with age, because the cells that produce medullas (osteoblasts) become less productive when forming new tissues and curing microcracks. Decrease of density considerably reduces resistance of porous tissues located at the ends of long bones and backbone vertebra. As a result different injuries occur, for example, multiple hipbone breaks for aged people or vertebra deformations and pain in backbone.

In 2003 the demand for supporting - motoric system implant materials in bone tissue engineering market reached 23.8 billion USD, but in 2013 increase might reach 39 billion USD. In 2004 just in the USA 1.5 million bone transplant procedures were carried out, the majority was middle - aged people, the number is expected to double in the next 25 years [1].

There has not yet been introduced a human bone tissue analogue, which would be a composite material with as complex structure as bone. However, creating materials, which can replace the mineral part of bone (porous bone), is today's reality. Medicine - material science problems are topical also in Latvia both in practical medicine and scientific research that is necessary to obtain new and competitive biomaterials in world market. Technologies, which use porous bioceramics, develop and necessitate elaboration of new porous ceramic production methods and improvement of the current methods.

The Objective of Research and Tasks

The objective of research is to produce porous calcium phosphate ceramics and investigate the impact of pore forming additives on structure and qualities of bioceramic materials, using different methods for obtaining porous ceramic materials.

According to the objective the following tasks were defined:

- formation of porous ceramic materials, using methods adapted from literature sources, considering different functional application restrictions of porous ceramics;
- research, analyses and establishing regularities of the obtained material structure and qualities, depending on pore forming additives used and methods of obtaining porous ceramics;
- production of sample series for clinical experiments *in vivo* (live organisms) research and ceramic samples *in vivo* evaluation;
- to forecast application options of the obtained porous ceramic materials.

Scientific Novelty of the Work

Scientific novelty of the study is establishing the regularities of interaction between the implant material, pore foaming agents, and the applied technology. The ceramic implant material - synthesized and commercial calcium phosphate and various pore forming additive (ammonium hydrogen carbonate, gelatine, starch, chitin, lycopodium, the polymer precursor) interaction and studied their composition - structure and properties change patterns have been determined experimentally using a variety of porous ceramic materials, experimental techniques.

Practical Importance of the Work

The results obtained in the study make it possible to obtain and to predict the porosity of calcium phosphate implant materials, the size, structure and properties of pores corresponding to the required medical applications.

Approbation of the Results of the Work:

The main results of research have been set out in the following publications (1.,2.,5. and 8.) and conference theses (3.,4.,6. and 7.):

1. R. Serzane, J. Locs, L. Berzina-Cimdina, R. Sadretdinovs. Development of porous ceramics by lycopodium using uniaxial pressing and sintering. Processing and Application of Ceramics, 2010, 4(*4*), 231-235.
2. I. Salma, M. Pilmane, A. Skagers, J. Vetra, G. Salms, L. Berzina-Cimdina, R. Serzane. Early Morphofunctional Response of Contact Tissue after Intraosal Implantation in Rabbit Jaw of Pure Synthetic Hydroxyapatite (HAp) Bioceramic Materials and HAp Saturated with Lidocaine. Stomatologija, 2009, 11(*4*), 113-118.
3. R. Serzane, J. Locs, I. Freimanis, I. Salma, L. Berzina-Cimdina. Porous Calcium Phosphate Ceramic Implants. Stomatologija, Baltic Dental and Maxillofacial Journal, 2009, 6, 44.
4. I. Salma, M. Pilmane, J. Vetra, A. Skagers, R. Serzane. Transforming Growth Factor β (TGFβ) In Contact Tissue to Synthetic Hydroxyapatite (HAp) Implants. Stomatologija, Baltic Dental and Maxillofacial Journal, 2009, 6, 40-41.
5. L. Berzina-Cimdina, R. Serzane, I. Salma, M. Pilmane, G. Salms, and A. Skagers. Drug infiltration in porous hydroxyapatite ceramic and tissue response. Integrated Ferroelectrics, 2008, 103(*01*), 66-71.
6. R. Serzane, Z. Irbe, L. Berzina-Cimdina. Porous Calcium Phosphate Bioceramic scaffolds. Abstracts 2 view, 8th World Biomaterials Congress, Amsterdam, The Netherlands, 2008 (CD-ROM).
7. R. Serzane, Z. Irbe, I. Salma, L. Berzina-Cimdina. Porous Calcium Phosphate Bioceramics. POROCER-2008 at Central Power Research Institute Bangalore; January 9-11th, 2008, 144-145.
8. R. Seržāne, L. Bērziņa-Cimdiņa, I. Šalma, K. Šalma. Porous calcium phosphate ceramics 3D-structures for biological cells scaffolds. Scientific Journal of Riga Technical University, Material science and applied chemistry, volume 15, 2007, pages 85-91.

Research results have been reported in the following local and international conferences:

1. J. Locs, R. Serzane, L. Berzina-Cimdina, R. Sadretdinovs. Development of Porous Bioceramics. "The Seventh Students` Meeting", SM -2009 Processing and Application of Ceramics, Novi Sad, Serbia, December 2-5th, 2009. Abstract book page No. 52.

2. R. Serzane, J. Locs, I. Freimanis, I. Salma, L. Berzina-Cimdina. Porous Calcium Phosphate Ceramic Implants. Conference 10th Joint Symposium Rostock - Riga, Latvia, May 07-10th, 2009.
3. I. Salma, M. Pilmane, J. Vetra, A. Skagers, R. Serzane. Transforming Growth Factor β(TGFβ) In Contact Tissue To Synthetic Hydroxyapatite (HAp) Implants. Conference 10th Joint Symposium Rostock - Riga, Latvia, May 07-10th, 2009.
4. R. Serzane, L. Berzina-Cimdina. Preparation of Porous Hydroxyapatite Ceramics. RTU 49th International Scientific Conference, Riga, Latvia, October 13-15th, 2008.
5. Z. Irbe, R. Serzane, J. Locs, L. Berzina-Cimdina. Development of Porous Bioceramics."7th ScanBalt Forum & ScanBalt Biomaterials Days"-Conference, Vilnius, Lithuania, September 24-26th, 2008, abstract pp.45.
6. R. Serzane, Z. Irbe, L. Berzina-Cimdina. Porous Calcium Phosphate Bioceramic Scaffolds. 8th World Biomaterials Congress, Amsterdam, the Netherlands. 28 May - 1 June, 2008.
7. L. Berzina-Cimdina, R. Serzane, I. Salma, M. Pilmane, G. Salms, and A. Skagers. Drug infiltration in porous hydroxyapatite ceramic and tissue response. FM&NT- 2008, Institute of Solid state Physics, Riga, Latvia, April 1-4th, 2008, abstract pp.178.
8. R. Serzane, Z. Irbe, I. Salma, L. Berzina-Cimdina. Porous Calcium Phosphate Bioceramics. Porocer-2008 and 71st Annual Session of the Indian Ceramic Society, Central Power Research Institute, Bangalora, India, January 8-11th, 2008.
9. R. Serzane, I. Salma, L. Berzina-Cimdina. Research of porous calcium phosphate bioceramics scaffolds. RTU 48th International Scientific Conference, Riga, Latvia, October 11-13th, 2008.
10. L. Bērziņa-Cimdiņa, R. Seržāne, K. Šalma. Porous calcium phosphate ceramics 3D-structures for biological cells scaffolds. RTU 47th International Scientific Conference, Riga, Latvia, October 12-14th, 2006.

LITERATURE REVIEW

The literature review spans the publicly available information during the period from year 1971 to 2011. It addresses the questions of calcium phosphate and composition, structure and properties of bone tissues. The study deals with features of pores and theoretical exposition of their classification. Porous ceramic materials, fillers, and the technological methods of obtaining these materials are described. The use of porous ceramics in medicine has been displayed, including analysis of biomaterials.

Literature data analysis and evaluation confirms that it is possible to improve complexity and bioactivity of constructions by combining progressive production technologies and new methods of material synthesis. Also, calcium phosphate ceramics, bioactive glass and glass ceramics have great potential for further use in regenerative medicine and tissue engineering. Suitable tissue engineering scaffold materials are still searched, having advisable degradation ratio and the required product and mechanical qualities to reach structure with advisable pore sizes, morphology, surface topography and bioactivity. Progress in material science, engineering, cellular and molecular biology and medicine can offer new solutions. The patient's own cells can be isolated and transplanted on scaffolds with advisable structure *in vitro* and, biologically stimulated, these cells can be multiplied, differentiated and grown to implant into human body to regenerate the unsound or the damaged tissues [2, 3]. Scientists still continue research on biomaterials' „regenerating" approach, based on fast biomaterial degradation and replacing with bone tissues, instead of replacing defect with an implant and suitable mechanical load.

Furthermore, concerning human body functions' regeneration, it is pretty obvious that when merging material sciences, biology and medicine, the current condition of research and development in the field of bioceramics materials faces the very start of solving new problems.

EXPERIMENTAL PART

The following raw materials were used to obtain porous bioceramics: ceramic structure forming components: hydroxyapatite powder synthesized at Riga Biomaterial Innovation and Development Center (RBIDC) of RTU, commercial hydroxyapatite powder $Ca_5(PO_4)_3OH$ (HAp) by Riedel - de Haën®, commercial tricalcium phosphate powder $Ca_3(PO_4)_2$ (β-TCP) by FLUKA). Porolone (foamed polyurethane) used as polymer precursor. Pore forming agents: ammonium hydrogen carbonate - NH_4HCO_3 (chemically pure, made in the European Union by ENOLA Ltd), lycopodium (lycopodium powder - lycopodium spores by JSC Riga Pharmaceutical Factory), gelatine (food gelatine made in the European Union), chitin (grinded chitin from crustacea shells), Aloja starch by ”Aloja Starkelsen” Ltd. Binding agents: proteins: dry protein powder and raw egg white, paraffin, poliol or glycerin (purity > 99.8%, by BIOVENTA SIA) and distilled water.

An important aspect determining the choice of the above pore forming agents is availability and economical use of material resources to study the impact of pore forming agents on structure and qualities of bioceramic materials.

On the basis of literature analyses and results of scientific publications, the following methods were used to obtain porous ceramics: polymer precursor impregnation, casting and uniaxial pressing.

1. Preparation of Samples by Polymer Precursor Impregnation Method, Drying and Burn - Out of Samples

Obtaining porous bioceramics by polymer precursor impregnation method is based on impregnating of polymer precursor - a porous matrix - with ceramic slips (composition of commercial HAp powder casting paste (slips) in water with a gel - linking (protein) agent) and fixing the resulting structure with heat treatment. After burning out the polymer precursor, the binder/ gel - forming agent, a porous ceramic structure is obtained.

With this method have been made two series of samples, they differ from the common ceramic technology with the added gel - forming agent/ binder (Fig. 1.).

Samples are formed by injection, using syringe, impregnating polymer precursors (square porolone pieces sized 10 x 10 x 10 mm) with reconstituted mass. The impregnated samples are dried at room temperature (19 ± 0.5°C) for 24 hours. After drying, samples are burned out for 1 hour at the temperature of 1250°C.

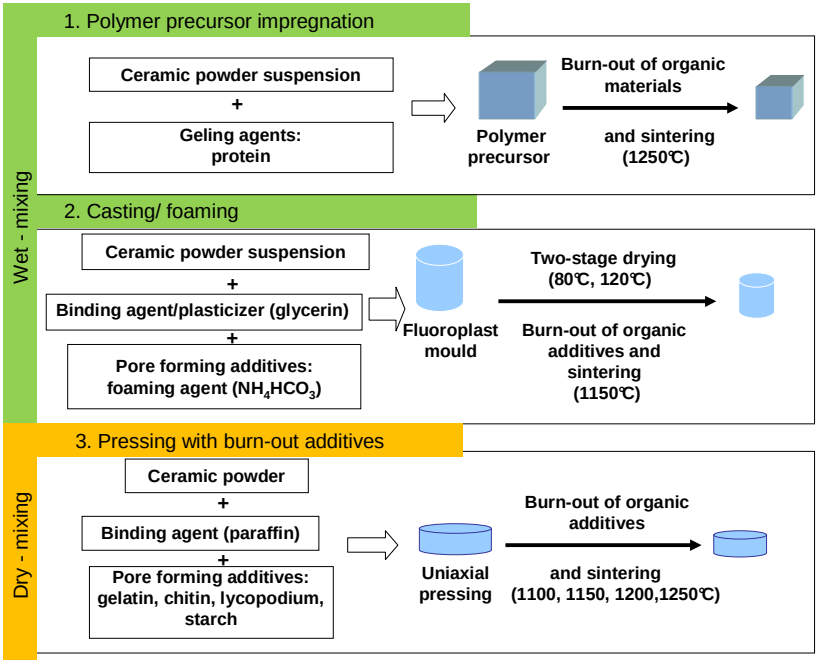


Fig. 1. Technologies of obtaining porous bioceramics

2. Preparation of Samples by Casting Method, Drying and Burn - Out of Samples

The method is based on the pore former NH_4HCO_3 which is added to the suspension and by decomposing creates hole spaces - pores. Pores in ceramic material are formed by decomposing the hard NH_4HCO_3 particles in the process of sample heat treatment.

Samples obtained with this method are made using casting technology (Fig. 1.).

Samples were formed by pouring reconstituted mass in cylindrical Teflon casts (Ø = 10 mm and h = 20 mm). After two - stage drying (80°C, 120°C) samples are removed from Teflon casts and burned out at constant temperature of 1150^{o}C for 2 hours, heating/ cooling carried out at rate of 5.5°C per minute.

3. Preparation of Samples by Uniaxial Pressing Method (powder compression), Drying and Burn - Out of Samples

Powder compression method for obtaining porous bioceramics is based on the separation of pore forming additives (gelatine, chitin, lycopodium, starch) in the process of sample heat treatment.

The mass is made with common technology that ensures making the samples with uniaxial pressing method (Fig. 1.).

HAp tablet form implants (Ø = 10 mm, h = 1.5 mm) produced using 0.35 g (± 0.01 g) mass are derived from the commercial HAp and synthesized HAp powder by dry powder pressing.

The prepared series of ceramics samples with different pore forming agent mass quantity (5%, 10%, 15%) are burned out at different temperatures 1100°C, 1150°C, 1200°C, 1250°C for 1 hour. Heating/ cooling carried out at rate of 5.5°C per minute.

4. *In Vivo* Research

Cooperation with the physicians of Riga Stradins University the first researches *in vivo* have been carried out. Experiments were conducted by inserting uniaxially pressed burned out porous HAp ceramic samples with the pore forming agent using gelatine 5% of weight. Samples before implanting in animal tissues - in rabbit lower jaw for two weeks, three months modified with lidocaine and dexamethasone. After the determined period re - implantation was carried out. Samples and bone tissues with surrounding soft tissues removed to perform morpho - functional investigation. Analyses carried out, using histology and immune histology methods.

The following equipment/devices and research methods were used for research:

- mixing of the components of the ceramic mass in certain proportions and sintering the mass using standard equipment.
- for determination the physical and chemical properties (volume mass, porosity) of the fired ceramic samples the method described in LVS EN 623-2 „Modern Technical Ceramic. Monolith Ceramic. General and Structural Properties. Part 2: Determination of Density and Porosity" is used. This method is based on Archimed's principle.
- high-temperature microscope *'EMO-1750/30-K'* used for ceramic powder sintering research;
- thermal contraction or manufactured samples of linear dimensional changes that occur in ceramic products during the heat treatment, the physical and chemical processes that can be expressed as a percentage of raw materials and sintered sample volume difference;
- X´Pert Pro (Panalytical) X-ray difractometer (XRD) used for crystalline phase analyses of samples. Additional samples analyzed by Fourier method, transformation infrared spectrometer (FT-IR) Scimitar 800 (Varian Inc.) used for investigation of chemical bonds in the range of 400 - 4000 cm^{-1}. Experiments conducted at dry air atmosphere using potassium bromide tablets technology;
- Leica MZ 16A stereomicroscope and scanning electron microscope (SEM) Mira/ LMU (Tescan) used for sample microstructure investigation; Macrostructure and microstructure research was carried out using SEM and stereomicroscope software;
- Material testing in bending and compression was done using a mechanical testing machine "Instron 430". Mechanic testing in four point bending was carried out with samples obtained with polymer precursor impregnation method, mechanic testing in pressing was carried out with samples obtained with polymer precursor impregnation method and casting method. The result was calculated as the mean value of five samples.

RESULTS AND DISCUSSION

In the study the properties of porous ceramic samples and their structure depending on the applied pore formers and obtaining methods, as well as from the sintering temperature have been explored and investigated.

Given that specific technology is necessary for producing ceramic materials for each pore formers, the study also examined the influence of these methods and their parameters on the structure and properties of ceramic materials.

Porous Polymer Precursor's Ceramics and Porous Ceramics Cast in Forms

Phase composition, microstructure and physically mechanical properties have been studied for the samples that are made by polymer precursor impregnation techniques and test series, made by casting techniques.

Features of the phase composition

Given that the phase composition depends on the technology and the structure of the used components and the properties of the materials, according to the obtained XRD and FT-IR data it was found, that in the sintering process of the ceramic precursor polymer samples at 1250°C as the only crystalline phases were detected HAp and β-TCP. The gel forming agent/ binder and polymer precursor decompose and burn out, without affecting or changing the chemical composition of the materials and the phase composition. According to the obtained XRD and FT-IR data it can be concluded that by sintering the ceramic samples cast in forms at 1150°C as the only crystalline phase is found HAp. The binders, pore forming additives burn out and/ or decompose affecting both the chemical composition of the material and the phase composition.

Characterization of the structure and evaluation of the qualities of porous ceramic polymer precursors and porous ceramic cast in forms

In the firing process the obtained porous polymer precursor's ceramics have built a netlike structure, similar to the precursor structure

of synthetic polymer. The porous polymer precursor ceramic is characterized by open, penetrating, interconnected mono - size pores of 450 - 500 μm (Fig. 2. A1), depending on the structures and sizes of the polymer precursor.

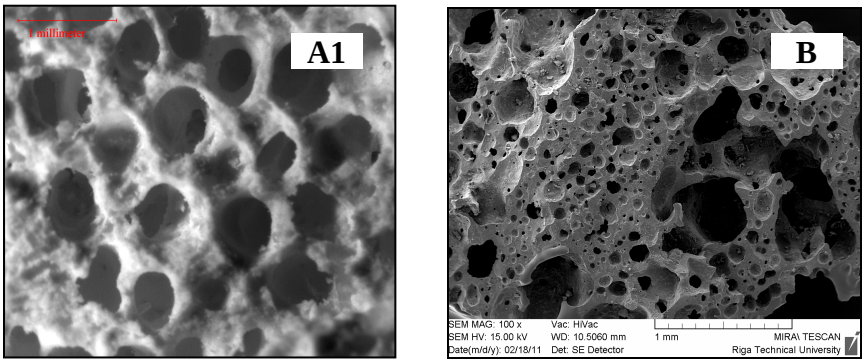


Fig. 2. Microstructure of porous ceramic precursor polymer (A1) and the ceramic cast in forms (B)

The arrangement of the pore channels in the ceramic is evenly distributed throughout the whole sample volume. Describing the morphology and the walls of the pores it was found that the pore walls of the experimental sample have a microporous structure, the size of mezo - sized pores are a few tens of microns. The grained boundary is attenuated, but it persists, the size of the grains is 2 - 5 μm. The pore structure of the examined bioceramic samples, obtained by polymer precursor impregnation method, is similar to the pore structure of cancellous bone.

By assessing the microstructure of the ceramic cast in forms, the studies have shown that the ceramic has porous, inhomogeneous volume structure with open, interconnected pores of different sizes (range 50 - 600 μm), the surface texture is rough, uneven (Fig. 2. B). On the sample surface and in the walls of the pores are mezo - sized (2 - 50 μm) and macro pores < 2 μm. The most of the wall area of the pores is covered by grains of rounded shape. The grain boundary is attenuated, however, it remains. Their sizes range from 200 nm - 1μm.

Table 1

Features and structure of porous polymer precursor ceramic and porous ceramic cast in forms

	Porous polymer precursor ceramic	Porous ceramic cast in forms
Sintering temperature, °C	1250	1150
Homogeneous structure	+	–
Open, penetrating, interconnected pores	+	+
Monosize pores	+	–
Macropores, μm	490 - 550	50 - 600
Open porosity, %	50 - 75	55 - 60
Volume mass, g/cm^3	1.36 - 0.56	1.10 - 1.02
Pressure strength, MPa	1.89 - 1.00	10 - 7
Elastic modulus between the stress level 0.1 un 0.2 MPa	45 - 38	–
Elastic modulus between the stress level 1.0 un 1.1 MPa	–	135 - 118
Thermal shrinking, %	54 - 58	65 - 69

With the polymer precursor impregnation method (Table 1) it is possible to get samples with porosity up to 75% (50 - 75%) and the storage volume from 1.36 to 0.56 g/cm^3. As shown by the obtained results of the study the compressive strength at such porosity is 1.89 to 1.00 MPa and elastic modulus - 38 - 45 MPa. In determining the mechanical properties of the samples, the regularity is confirmed that the porosity, the size of pores and their arrangement in the volume of the material are important parameters that affect these properties. The low mechanical strength of the examined materials is due to their low apparent density and relatively high porosity. The thermal shrinkage of porous polymer precursor ceramic makes 54 - 58%.

With the casting method (Table 1) it is possible to get samples with porosity of 55 - 60% and the storage volume from 1.10 to 1.02 g/cm^3. The indicators of the compressive strength are 10 - 7 MPa and elastic

modulus range from 118 to 135 MPa. The large dispersion of the examined material is related to the inhomogeneous arrangement of the pores in the volume of the material and the sizes of multipores. The shrinkage of the ceramic after drying at 1150°C is 65 - 69%, which contributes to the size reduction of the pores and the channels connecting them and their necks. It can be explained by the fact that with the rise of the temperature the sintering becomes more intense that furthers the compaction of the particles. This is evidenced by the increasing shrinkage value. The applied casting technology does not ensure homogeneous pore formation throughout the whole volume of material.

Although the porous polymer precursors, as well as the porous ceramics cast in forms, have a large dispersion of parameters as well as on the breaking stress, as on the elastic modulus, which is a cause of very porous and inhomogeneous structure and geometrical inadequacy of the samples, it does not diminish the value of the experimental results and it is reasonable to conclude that the materials are applicable to the production of porous ceramic implants, such as fillers for preventing bone tissue defects, production of porous substrates and carrying out further researches with drug distribution and cellular studies.

Uniaxially Pressed Porous Ceramics

Porous ceramic microstructure (Table 2, Fig. 3.) and the physically mechanical properties are investigated for the series of samples made on the base of commercial and synthetic HAp with uniaxial pressing the phase compositions.

Features of phase's composition

After the obtained XRD and FT-IR data it has been stated that at the sintering temperature 1100 - 1250ºC the phase composition is characterized as crystalline phase - hydroxyapatite. The binding agent and pore formers added in the sintering process (5%, 10%, 15%) do not affect and not change the chemical and phase composition of the materials. The peaks of the synthetic HAp - based ceramic compositions, as well as the difractogrammes of the synthesized HAp powder are less intense and wide, which shows fine crystallization of the samples.

Characterization and evaluation of the qualities of the structure of uniaxially pressed porous ceramic

Evaluating the surface structure of uniaxially pressed porous ceramic composition on the base of commercial and synthetic HAp, where starch, lycopodium, gelatine and chitin are used as pore forming additives in the contents of mass, it can be concluded that a rough, inhomogeneous, surface structure has been obtained (Fig. 3.).

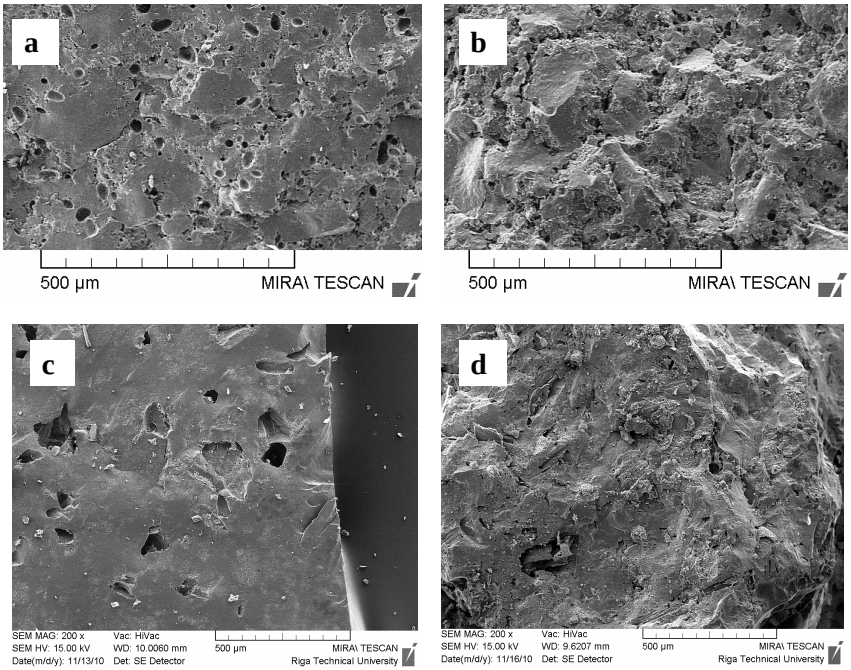


Fig.3. Microstructure of porous ceramic, with a) starch, b) lycopodium, c) gelatine, d) chitin as pore formers

Under the influence of the used pore former pores of different size and forms have been found in the microstructure of the ceramic, that depend on the amount of the pore forming mass, the sintering temperature and the basic component of ceramic material - commercial or synthesized HAp.

Table 2

Characteristics of pores

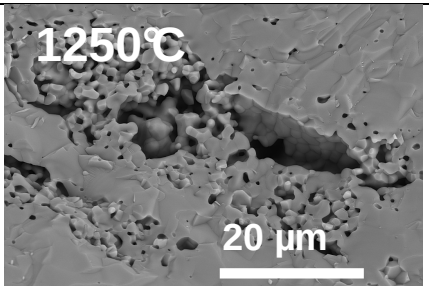
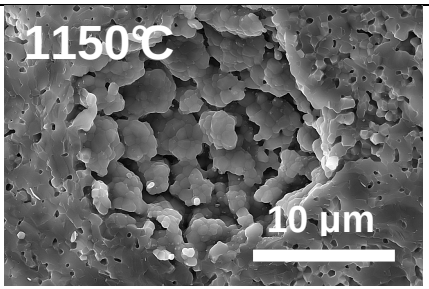
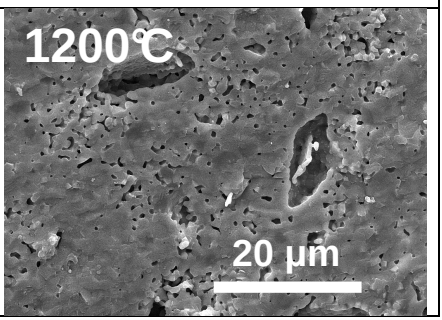
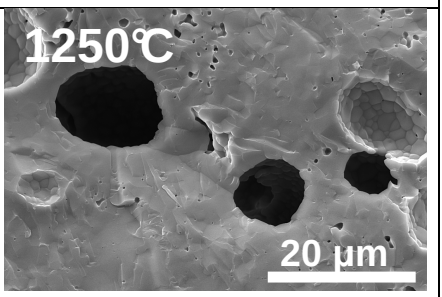
Pore former, its amount	Sintering temperatures 1100, 1150, 1200, 1250°C	Microphotographies: mezo - size pores and micropores
Gelatine 5 % of weight	Multi - sized pores of irregular forms: the size of macropores range 50 - 350 μm, mezo - sized pores - 2 - 50 μm, open, not penetrating pores, on the surface and in the walls of the pores are open, penetrating micropores < 2 μm.	
Lycopodium 5, 10, 15 % of weight	Mono - sized, round (globulus) pores, the size of mezo - sized pores 17 - 22 μm, open, not penetrating pores, on the surface and in the walls of the pores are open, penetrating micropores < 2 μm.	
Chitin 5, 10, 15 % of weight	Multi - sized pores of irregular forms: the size of macropores range 50 - 200 μm, mezo - sized pores - 2 - 50 μm, open, penetrating pores, on the surface and in the walls are open, penetrating micropores < 2 μm.	

Table 2 continuation

Pore former, its amount	Sintering temperatures 1100, 1150, 1200, 1250°C	Microphotographies: mezo - size pores and micropores
Starch 5, 10, 15 % of weight	Multi - sized, roundish pores, the size of mezo-sized pores are between 2 - 45 μm. Open, not penetrating and partially channel building pores. On the surface and in the walls are open, penetrating micropores < 2 μm.	

The microstructure of porous ceramic is shown as an illustrative example that is obtained with the pore forming additive - lycopodium. The ceramic microstructure depends on the amount of the added forming and the sintering temperature (Fig. 4.).

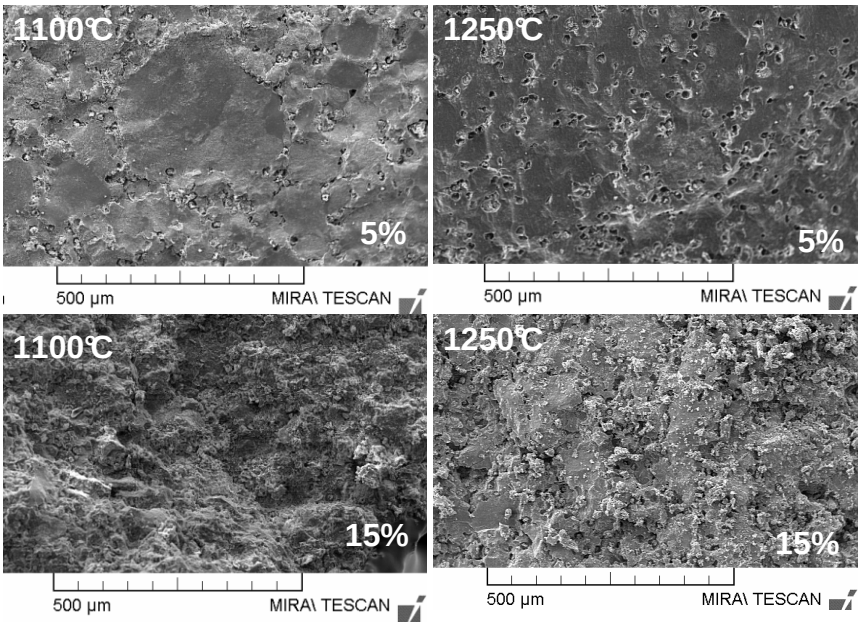


Fig.4. Microstructure of porous ceramic where lycopodium is used as pore former

Raising the sintering temperature from 1100 to 1250ºC furthers enlargement of the mezo - sized pores, growth of grains and diminishing of the total pore surface. The result of these processes is sintering (Fig. 4.).

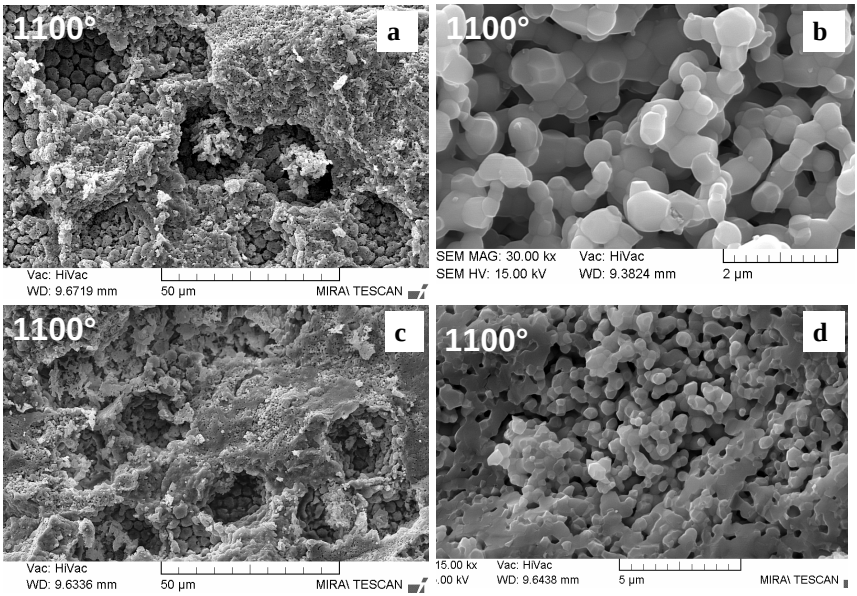


Fig. 5. Microstructure of porous ceramic on the base of the commercial HAp (a, b) and the synthesized HAp (c, d) with lycopodium as pore former

Compared to the sample, where the pore forming additive used licopodium, microstructure of commercial and synthesized HAp - based (Fig. 5.) is observed, that sintering the samples on the commercial HAp base at 1100ºC boundaries between the grains are attenuated, but they still exist. The structure of samples on synthesized HAp base has densely sintered areas of grains because the synthesized HAp powder is fine crystallized (with the size of agglomerate particles ~ 10 - 50 µm) (Fig. 5.).

Porosity, volume mass and mechanical strength of the ceramic composition, obtained with lycopodium as pore forming additive (5, 10, 15 wt %) depended on the composition of the pore former, the added amount and the sintering temperature (Fig. 6.).

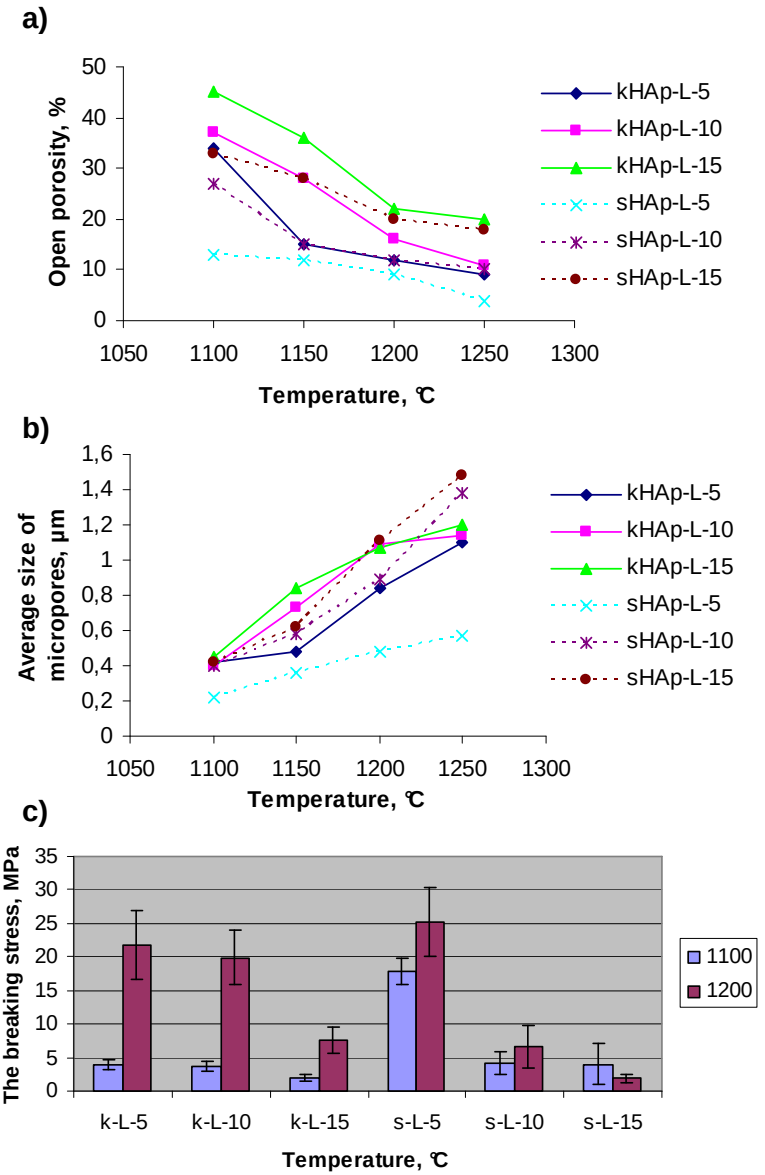


Fig. 6. Open porosity of porous ceramic obtained with lycopodium(a), average size of micropores (b) and the breaking stress at four point bending (c) depending on the sintering temperature.

By raising the sintering temperature from 1100 to 1250ºC the open porosity of samples diminishes and their volume mass increases. For the compositions based on commercial HAp the enlargement of the volume mass is 1.49 - 2.58 g/cm^3, but for the synthesized HAp - 1.58 - 2.89 g/cm^3.

The greater the amount of added lycopodium, the higher the open porosity (Fig. 6. a, samples s - synthesized HAp, k - the commercial HAp). Diminishing the amount of the added lycopodium from 15% to 5% and increasing the sintering temperature in the range of 1100 - 1250°C, result in open porosity decrease. The most intense reduction of open porosity is observed in the temperature range between 1100 - 1200°C; it can be explained with the compaction of crystalline particles in the sample, filling of the open pores, because in the sintering process the pores are forced out of the sample volume. Using 5% lycopodium as pore forming agent in synthesized HAp ceramic compositions, the changes of the open porosity in the temperature range 1100 - 1250°C are negligible and decrease from 13% to 4%.

The relationship between the average size of micropores, the quantity of pore forming agent - lycopodium and the sintering temperature (Fig. 6. b) is experimentally observed. Increasing the sintering temperature from 1100 to 1250°C and reducing the amount of the added lycopodium by 15 - 5 %, the average size of micropores decreases.

Microstructure of ceramic materials, their porosity (amount, sizes of the pores) affect most directly the strength of these materials. Determining the breaking stress at four point bending (Fig. 6. c), they are higher at the compositions that were sintered at 1200°C. The greater the amounts of the added lycopodium in the ceramic mass, the greater the porosity, but lower the bending resistance. Reducing the amount of the added lycopodium till 5 mass %, the bending strength increases 13 times in comparison with 15%. When comparing the mechanic values in bending we can conclude that the reason of decrease of strength is different sintering and changes in porosity. When comparing the mechanical performance of bending, it can be concluded that the cause of diminishing the mechanical qualities are the differences in ceramic sintering, the increase of porosity and the large sizes of micropores. Experimental results suggest that the ceramic bending strength increases by increasing the firing temperature, which can be explained by the

increase in density and porosity decrease. By loading the samples we obtain regularities of quasi linear stress strain.
As an example are demonstrated ceramic compositions based the commercial HAp obtained by heat treatment of 1200°C with the highest amount of pore forming additives (15wt %) in the moulding (Fig. 7.).

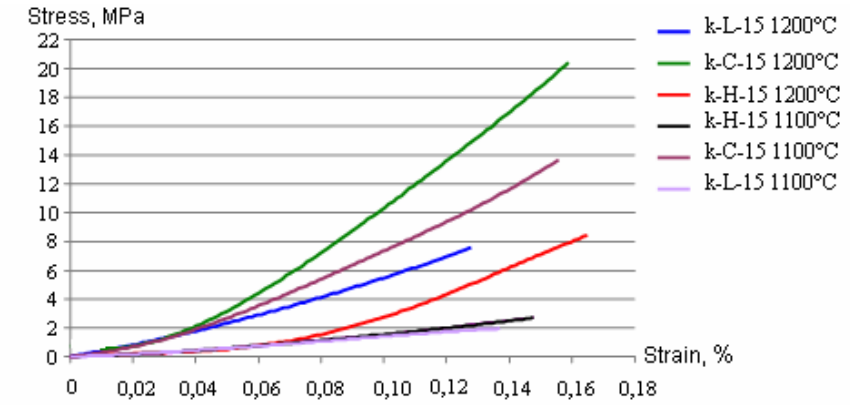


Fig.7. Standard quasi-linear stress-strain curves of samples of uniaxially pressed porous ceramic (pore forming additives L- lycopodium, C- starch, H- chitin)

Although porous uniaxially pressed ceramics have a large dispersion of the breaking stress and the parameters of elastic modulus, it does not reduce the importance of the sample results and it can be concluded that they are applicable to the production of porous ceramic implants as fillers of bone defects, production of porous substrates and further experiments with the drug distribution and cell studies.
All effects of pore formers which are introduced into ceramic mass on the structure and properties of the biomaterial in the drying process are similar, only the morphology of structure (pores, crystalline grains, boundaries) (Table 2) and the numerical values of properties change (Table 3).

In the results of the effect of the examined pore forming additives - lycopodium, starch and gelatine - it is possible to obtain hydroxyapatite ceramic, which would be perspective for controls of drug infusion and discharging systems, because the elaborated biomaterials have porosity up to 45% and mechanical properties suited for particular application.

Characteristics of uniaxially pressed porous ceramics Table 3

Physical- mechanical properties						
Sintering temperature°C		1100 - 1250		1100 and 1200		
Pore forming agent	Pore former mass %	Open porosity %	Volume mass g/ cm^3	The breaking stress at four point bending, MPa	Elastic modulus between the strain level 1.0 and 1.5 MPa	Elastic modulus between the strain level 2.0 and 3.0 MPa
Compositions with the ceramic base component – commercial HAp						
Lycopodium	5 - 15	45 - 9	1.5 - 2.6	1.97 - 21.81	3123 - 8982	-
Starch	5 - 15	26 - 2	1.9 - 2.8	9.93 - 36.28	3415 - 12400	-
Gelatine	5	32 - 12	1.9 - 2.5	5.48 - 6.68	3484 - 4803	-
Compositions with the ceramic base component – synthesized HAp						
Lycopodium	5 - 15	33 - 4	1.9 - 2.9	1.85 - 25.28	-	4554 - 12490
Starch	5 - 15	18 - 2	2.2 - 2.9	7.61 - 20.71	-	9240 - 13720
Gelatine	5	11 - 3	2.6 - 2.8	11.06 - 18.80	-	10710 - 11490

Researches of Uniaxially Pressed Porous Ceramics *in Vivo*

The first studies were performed with uniaxially pressed porous ceramic samples, where gelatine making 5% of weight was applied as pore former. The test results indicate various growth factors (growth factors are proteins that are secreted by cells and act on certain cells, causing a specific response by influencing extracellular matrix synthesis, cell division, differentiation, proliferation, migration and death), which depend on drugs, which impregnated implants. After the implantation there was no inflammatory reaction (Fig. 8.). The study shows that the samples containing lidocaine are less apoptosis after implantation (apoptosis is defined as a genetically programmed mechanism of cell perish without signs of inflammation from the surrounding tissues, which differs from morphology and biochemistry of cell necrosis) than in the control group 3 months later after the implantation. The figure shows a brown core - an apoptotic core.

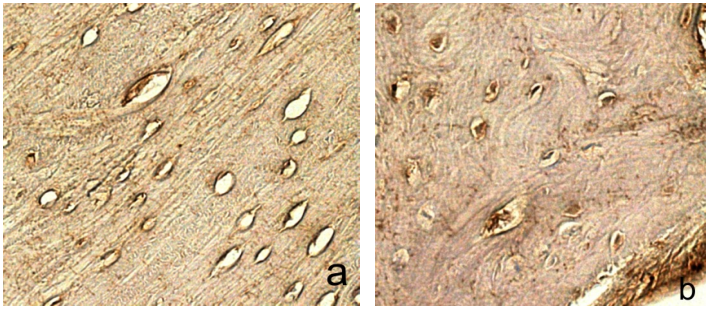


Fig. 8. Apoptosis in rabbit lower jaw three months after HAp implantation with dexamethasone (a) and with lidocaine (b). Slight apoptosis in samples with dexamethasone; moderate apoptosis in samples with lidocaine. TUNEL, IMH x250.

The obtained results show that biomaterials with good biocompatibility do not cause inflammation in tissues and after the implantation a thin connective tissue capsule is created in soft tissues without presence of inflammatory cells.

CONCLUSIONS

1. Parameters of technological methods on how to obtain porous ceramic depending on different foaming applications and their impact on ceramic materials, microstructure and physical - mechanical properties have been developed.
2. It has been established that the pore forming additives decompose and burn out in the heat-treatment process and do not react chemically with the ceramics - forming the basic components - calcium phosphate and do not affect their initial phase composition.
3. Porous polymer precursor ceramic is characterized by a homogeneous microstructure, penetrating, mono - sized interconnected pores (450 - 500 μm). The pore structure of the studied sample pore structure that was obtained by polymer precursor impregnation method is similar to the pore structure of the cancellous bone.
4. It has been stated that the microstructure of the ceramic which is cast in fluor plastic shapes, with NH_4HCO_3 as the foaming mass, has inhomogeneous microstructure with penetrating, multi - sized interconnected pores (50 - 600 μm), the surface texture is rough and heterogeneous, the size of grains - 200 nm - 1 μm, the boundaries are attenuated, the size of the pores depends on the size of pore forming particles.
5. Studies on the impact of foaming materials - starch, lycopodium, gelatine and chitin on uniaxally pressed ceramic microstructure showed that the fired (1100 - 1250°C) ceramics are characterized by rough inhomogeneous surface structure, in the microstructure are registered pores of various sizes (micro, mezo, macro) and shapes (rounded, irregular globule type), which depend on the quantity of added pore formers, the sintering temperature and the basic components of the ceramic material (commercial and synthesized HAp).
6. Porosity of ceramic compositions, volume weight and mechanical strength depend on the foaming composition, the added amount and the sintering temperature.
7. By raising the drying temperature of ceramics from 1100 to 1250°C, the open porosity of samples decreases, increasing their density and mechanical strength.

8. All effects of pore formers which are added to the ceramic mass on the structure and properties of the biomaterial in the sintering process are similar, only the morphology of structure (pores, crystalline grains, boundaries) and the numerical values of properties change.
9. *In vivo* research approved that hydroxyapatite with gelatine as pore former has a high compatibility, does not cause tissue irritation, and after its implantation in the soft tissues a thin connective tissue capsule form without irritation cells.
10. Properties regulating their use in reconstructive surgery determined by the purpose. The obtained ceramic materials are applicable as porous substrate to propagation of cells, to fillings for preventing bone defects, to producing drug infusion systems etc.

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