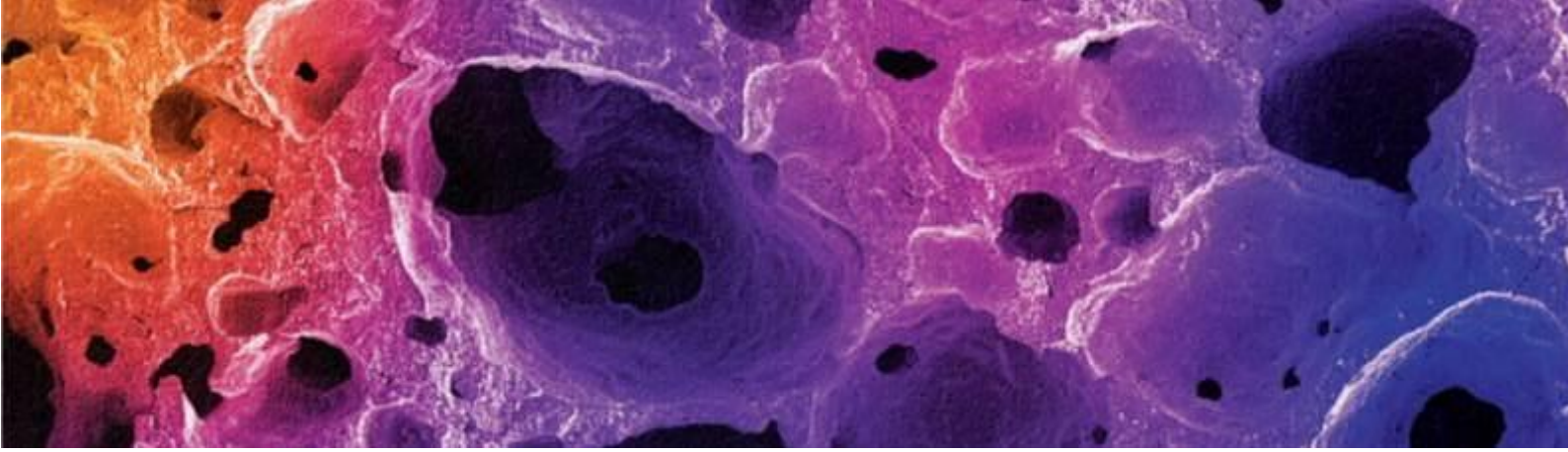

Rīgas Stradiņa universitāte

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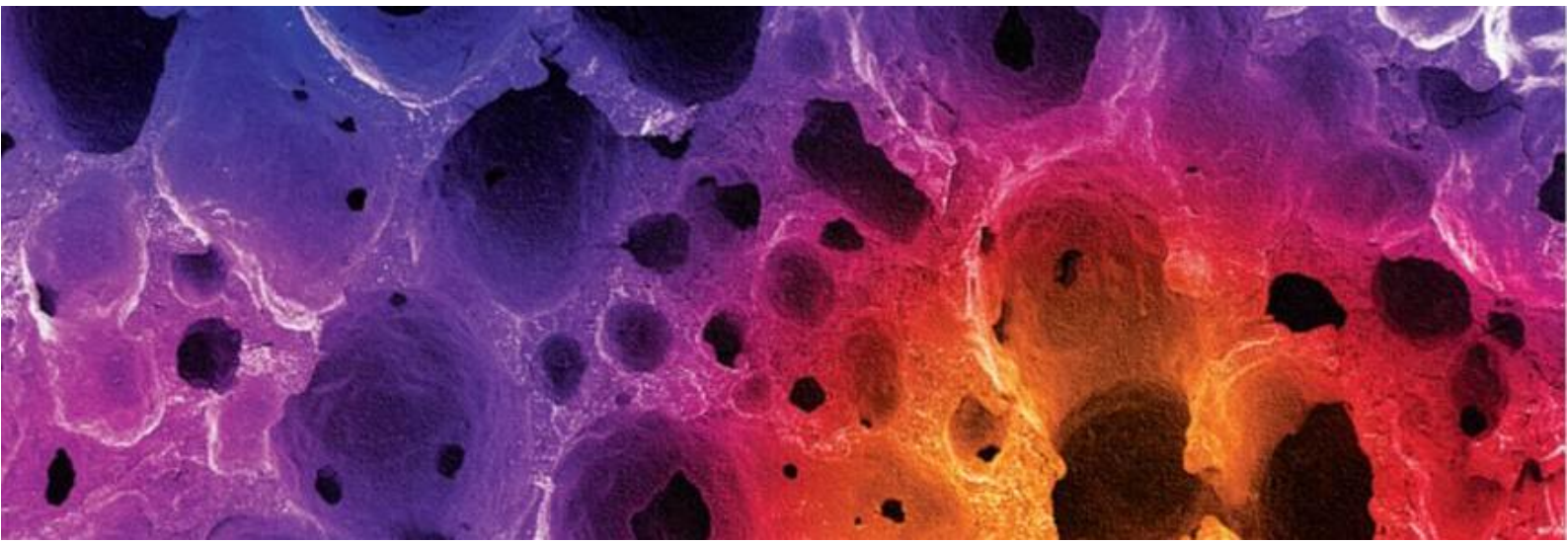
Rīgas Tehniskā universitāte RBIAC

BIOCERAMICS AND CELLS FOR REINFORCEMENT OF BONE

October 18-20, 2012, Riga, Latvia



ERAF
EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS

Dear friends and colleagues!

It is our pleasure to welcome you at the Symposium which is held to share, to reflect and to learn from one another new trends in the biomaterial science, bone tissue engineering, experimental and clinical work devoted to the possibilities of replacing autologous and other bone grafts by bioceramic scaffolds loaded with different cells, including stem cells.

We appreciate the participation of high-ranking scientists from France, Germany, Lithuania, Russia, and Spain. Thank you for coming and sharing your experience!

In Riga Rudolfs Cimdins Biomaterial Innovation and Development Centre at Riga Technical University and Riga Stradiņš University have long-standing cooperation in technologies for the development of new biomaterials to substitute bone tissue damaged due to diseases, injuries and after surgery, as well as testing these materials in animal research and in clinical use, mainly in oral and maxillofacial surgery.

Riga, the capital of Latvia, also called the city of inspiration, officially was founded in 1201. The architecture of the historic centre of Riga may boast of representing all architectural styles characteristic of Northern Europe, from Gothic to Modernism. The historic centre of Riga is a UNESCO World Heritage Site. Today Riga is a dynamic and pulsating city. Inhabitants of Riga almost continuously organize and participate and discuss different events. You only have to keep track of the time and the venue well in advance. Whether the aim of the event was achieved and whether it was worth participating in it is left open for each participant to answer, It concerns this Symposium as well.

We wish you to have a nice time here, in Riga, and we hope that during this Symposium old ties of collaboration will be renewed and new ones developed.

Welcome to Riga, Latvia, October 18–20, 2012!

Sincerely yours,

Ilze Akota
Professor,
Vice-Rector for Education Riga Stradiņš University

Liga Berzina-Cimdina
Professor, Riga Technical University,
Director, Rudolfs Cimdins Riga Biomaterial Innovation
and Development Centre, Riga Technical University

ORGANIZING COMMITTEE:

Andrejs Skagers	Department of Oral and Maxillofacial Surgery, RSU Institute of Stomatology
Liga Berzina-Cimdina	Rudolfs Cimdins Biomaterials Innovation and Development Centre, Riga Technical University
Girts Salms	Department of Oral and Maxillofacial Surgery, RSU Institute of Stomatology
Agnese Berzina	Development and Projects division, Riga Stradiņš University

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Ieva Greitane, Jekabs Salms	RSU Institute of Stomatology; Room 318, Dzirciema Street 20, Riga LV-1007; e-mail: askagers@latnet.lv; igreitane@latnet.lv; gsm: (+371) 27028427; (+371) 27065329; phone: (+371) 67455523

ACCOMMODATION:

Hotel Garden Palace, Grecinieku Street 28, LV 1079 (Old Town), phone (+371) 67224650; info@hotelgardenpalace.lv

Transfer from the hotel to the symposium venue on Friday and Saturday at 8:15

VENUE:

18.–19.10.2012.	Institute of Stomatology, Riga Stradiņš University, Dzirciema Street 20, Riga
20.10.2012.	Rudolfs Cimdins Biomaterials Innovation and Development Centre, Riga Technical University, Pulka Street 3/3

MEDGRUPĖ

SIROWA
Riga Tallinn Vilnius Helsinki

DENTOFLEX SCHUMANN

PROGRAMME

18.10.2012 **RSU INSTITUTE OF STOMATOLOGY, Dzirciema Street 20**

17:30–19:00 Registration
18:00 Get-together party

19.10.2012 **RSU INSTITUTE OF STOMATOLOGY, Dzirciema Street 20**

8:00 Registration

8:45 Opening – I. Akota, Vice-Rector for Education, Riga Stradins University (*Latvia*)

1st session Chairs: G. Salms (*Latvia*) S. Grybauskas (*Lithuania*)
9:00–9:30 Basic principles of biocompatibility: semantic and biomedical approach
J. Vetra (*Latvia*)
9:30–10:00 Osteoporosis and bioceramics: a positive future
D. Shepherd (*United Kingdom*)
10:00–10:30 The use of scaff olds with vegf dna-plasmid for bone regeneration in a critical size defect model
R. Deev (*Russia*)

10:30–11:00 Coffee break

2nd session Chairs: A. Yaremenko (*Russia*) D. Shepherd (*United Kingdom*)
11:00–11:20 Research on stem cells in Latvia
E.Jakobsons (*Latvia*)
11:20–11:40 Past and present of bioceramics in Latvia: experimental animals and clinical use
A. Skagers (*Latvia*)
11:40–12:00 Bioceramics in orthognathic surgery
S. Grybauskas (*Lithuania*)
12:00–12:20 In vitro evaluation of biomaterials synthesized in Riga Technical University
N. Romanchikova (*Latvia*)
12:20–12:40 Bone defect management in orthopaedic surgery with application of biphasic calcium phosphate
S. Petronis (*Latvia*)

12:40–13:40 Lunch

3rd session Chairs: M. Pilmane (*Latvia*) R. Deev (*Russia*)
13:40–13:55 Morphological evaluation of bone after implantation of hydroxyapatite and biphasic ceramic bone substitutes
I. Salma (*Latvia*)
13:55–14:10 Evaluation of sinus-lift enforced with biomaterials by cone-beam computed tomography
L. Neimane (*Latvia*)
14:10–14:25 Analysis methods of antibacterial effi ciency of retainers impregnated with antibiotics
I. Skadiņš (*Latvia*)
14:25–14:40 Morphogenesis around hap bioceramic implants loaded with authologous mesenchymal cells after implantation in subcutaneous tissue of rabbits
I. Cakstina (*Latvia*)

14:40–15:10 Coffee break

4th session Chairs: J. Kroica (*Latvia*) V. Korolev (*Russia*)
15:10–16:40 Poster presentation:

Time-depending tissue changes after implantation of biomaterials covered by stem cells in subcutis and subperiostaly of experimental animals
M. Pilmane, A. Skagers, G. Salms, I. Salma (*Latvia*)

Human gingival fibroblast behavior on modified titanium surfaces
V. Sabaliauskas (*Lithuania*)

Expression of bone regeneration proteins in rabbit bone tissue after the implantation of various bioceramic materials
J. Vamze, M. Pilmane, A. Skagers (*Latvia*)

Autologous fi brin/bioceramic scaffold for bone tissue engineering
I. Salma, A. Skagers, M. Pilmane, V. Zalite (*Latvia*)

In vitro and in vivo study of bacterial adhesion and colonisation on different biomaterials
A. Reinis, J. Kroiča, J. Vētra, M. Pilmane, A. Stunda, A. Pūra, D. Loča, A. Dubnika, I. Skadiņš, D. Rostoka *(Latvia)*

18:00 Gala dinner (invitations available at the registration, charge – LVL 10.00)

20.10.2012. **RUDOLFS CIMDINS BIOMATERIALS INNOVATION AND DEVELOPMENT CENTRE OF RIGA TECHNICAL UNIVERSITY, Pulka Street 3/3**

8:30 Registration

8:45 Opening – T. Juhna, Vice-Rector for Research, Riga Technical University

1st session Chairs: E. Burkel (*Germany*) J. Vetra (*Latvia*)
9:00–9:30 Determing a clinical relevant bone engineering method. An all in one study in nude mice
P. Weiss (*France*)
9:30–10:15 New approaches for development of osteoplastic materials
R. Deev (*Russia*)
10:15–10:45 Calcium phosphate bioceramics for reinforcement of bone
V. Komlev (*Russia*)
10:45–11:15 The role of silicon substitution on βTCP ceramic
E. Lopez – Cabarcos (*Spain*)

11:15–11:45 Coffee break

2nd session Chairs: P. Weiss (*France*) E. Lopez – Cabarcos (*Spain*)
11:45–12:15 Advanced Titanium alloys and foams for orthopedic application
E. Burkel (*Germany*)
12:15–12:35 Integration of calcium phosphate bioceramics in maxillary sinus floor
G. Salms (*Latvia*)
12:35–13:20 Engineering of bioceramics in Latvia: Technologies and preclinical assessment
L. Berzina-Cimdina (*Latvia*)

13:20–14:20 Lunch

3rd session Chairs: I. Bozo (*Russia*) J. Locs (*Latvia*)
14:20–15:30 Poster presentation:

Synthesis methodology and investigation of calcium phosphate biomaterials for bone tissue replacement in regenerative medicine
K. Salma-Ancane, A. Dubnika, L. Stipniece, M. Sokolova, N. Borodajenko (*Latvia*)

Mechanically stronger bioactive calcium phosphate glass and glass-ceramic
A. Stunda-Zujeva, G. Krieke, L. Poca, L. Berzina-Cimdina (*Latvia*)

Extrusion and thermal treatment of TiO_2 ceramic for various application
A. Pura, K. Rubenis, I. Narkevica, J. Ozolins, J. Locs (*Latvia*)

Development of porous calcium phosphate ceramics in Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre
V. Zalite, M. Sokolova, I. Freimanis, J. Locs, L. Berzina-Cimdina (*Latvia*)

Improvement of calcium phosphate cement cohesion using liquid phase additives
Z. Irbe, D. Vempere, D. Loca, L. Berzina-Cimdina (*Latvia*)

Scale – up and wet precipitation synthesis of calcium phosphates
I. Kreicbergs, M. Sokolova, A. Putnins, S. Bulina (*Latvia*)

Preparation of controlled release drug delivery systems based on calcium phosphates
D. Loca, A. Dubnika, V. Zalite (*Latvia*)

Recombinant HBcAg production in *E. coli*
K. Rugele, J. Vanags, A. Dislers (*Latvia*)

Investigation of silver doped hydroxyapatite
A. Dubnika, D. Loca, L. Poca (*Latvia*)

15:30 Final discussion. Concluding remarks.

October 19th, 2012
ORAL PRESENTATIONS

BASIC PRINCIPLES OF BIOCOMPATIBILITY: SEMANTIC AND BIOMEDICAL APPROACH

J. Vetra
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OSTEOPOROSIS AND BIOCERAMICS: A POSITIVE FUTURE

D. Shepherd

Department of Materials Science and Metallurgy,
University of Cambridge, United Kingdom
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Osteoporosis is a bone disease that leads to a dramatic reduction in bone mineral density (BMD). The loss of BMD makes the sufferer more susceptible to fractures with a particular risk for the hip, wrist joints and the lower spine. In 2000 it is estimated that there were approximately 0.9 million osteoporotic hip fractures. The total direct costs associated were estimated at €31.7 Billion which is expected to rise to €76.7 Billion by 2050 [1].

These hip fractures generally result in replacement of the natural joint with an implant but this is often associated with a loss of function and independence of survivors with a fair proportion moving into care homes within a year of the fracture [2]. Some patients will require revision surgery due to aseptic loosening and deep infection. Hydroxyapatite coated hips however, have the best survival rates [3].

The aim of this talk is to discuss the use of substituted ions into the hydroxyapatite lattice to increase the longevity of the implants further. These ions must induce one or more of the following responses; positive osteoblastic response resulting in more bone being laid down; down regulation of osteoclastic resorbtion; antibacterial properties.

Work on zinc substituted hydroxyapatite (ZnHA) has shown it to be an ideal candidate for this with it meeting all three requirements. Work by this author indicated that up to 0.65wt% zinc substitution has led to a significant reduction in the number and subsequent activity of the osteoclasts.

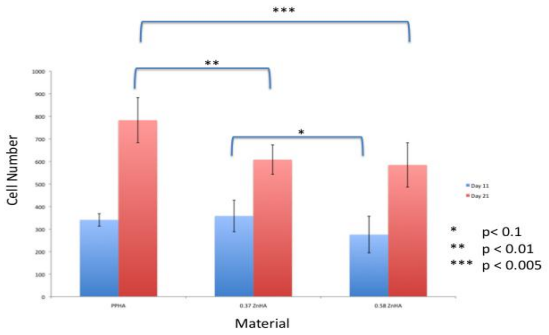
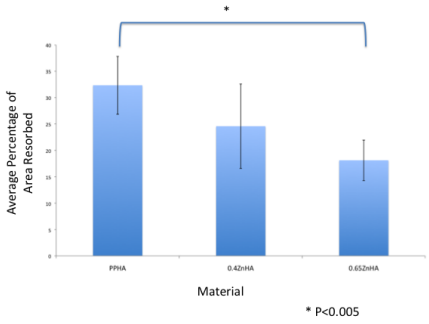


Figure 1. Graph showing effect of zinc on the number of osteoclasts after 11 and 21 days.

Figure 2. Effect of zinc on the resorption activity of osteoclasts after 21 days.



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THE USE OF SCAFFOLDS WITH VEGF DNA-PLASMID FOR BONE REGENERATION IN A CRITICAL SIZE DEFECT MODEL

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INTRODUCTION

At present, maxillofacial surgeons and dentists are increasingly faced with the necessity to replace various bone defects among different groups of patients. This is due to several factors: the increase in the number of injuries and ostepenia associated with urbanization, progress of implantology, pathological bone tissue resorption associated with alveolysis, periodotitis, natural need for bone tissue in reconstructive operations and introduction of new methods of treatment.

MATERIALS AND METHODS

The study investigated the effects of vascular endothelial growth factor (VEGF) DNA-plasmid non-covalently bound to different scaffolds for bone regeneration in a rat cranial and ilium critical size defects. Two groups of two different xenogenic scaffolds were generated with VEGF DNA-plasmid and implanted within 6–7 mm rat cranial and ilium critical size defect. At 15, 30, 45 and 60 days implants were retrieved and evaluated by computed tomography and histological scoring analysis.

RESULTS

The histological analysis and CT results showed larger and faster blood vessel formation, significantly higher bone formation de novo in the groups with scaffolds modified by VEGF DNA-plasmid in the dynamics of 15, 30, 45 and 60 days.

CONCLUSION

The experiment in vivo showed the great potential of scaffolds modified by VEGF DNA-plasmid to form organotypic bone regenerate in a critical size defect model and to decrease the time of regeneration. The use of such biomaterials in maxillofacial surgery may be prospective for bone augmentation and replacement in different types of bone defects.

RESEARCH ON STEM CELLS IN LATVIA

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PAST AND PRESENT OF BIOCERAMICS IN LATVIA: EXPERIMENTAL ANIMALS AND CLINICAL USE

A. Skagers

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Initiative for in vivo and clinical evaluation of bioceramic materials synthesized in Riga Technical University, Laboratory of Biomaterial Research, came from Rudolfs Cimdins and Liga Berzina–Cimdina in the early 1990s. The first step was studies on biocompatibility of different calcium phosphate bioceramic materials after implantation in experimental animals. After confirmation of good biocompatibility through evaluation of non-specific and specific reactogenicity clinical studies were started by application of granules and blocks of pure Hap, TCP and biphasic calcium phosphate bioceramics for reinforcement of atrophic jaws to increase the size of alveolar bone for insertion of dental implants, also for contourplasty in facial skeletal deformities. Now we have more than 300 clinical cases of use of Hap, Hap/TCP mainly in maxillary sinus floor elevation (sinus lift). Some data on reactogenicity of materials were elucidated and positive clinical results obtained. Integration of bioceramic implants was different in different tissue environment as encapsulation in soft tissue, chondro- and osteogenesis in subperiostal space and osseointegration in cases of intraossal implantation. Porous synthetic Hap ceramic granules implanted in wounds of rabbit ear cartilage in part of non-specific reactogenicity has no more active inflammatory response as in control wounds. In part of specific reactogenicity there was more active differentiation and proliferation of chondroblastic cells which may be the result of chondroinductive potency. The confirmation for bioactivity of synthetic porous Hap bioceramics as release of endogenous bone growth factors (TGFβ), biodegradation by osteoclast-like cells as in bone remodeling and increase of mineral density in contacting atrophic jaw bone was obtained.

At present experimental studies on reactogenicity of different calcium phosphate bioceramic materials are going in part of bioceramic materials loaded with drugs, autologous mesenchymal cells, and microbes. In clinical application the main field is oral and maxillofacial surgery, started also in orthopedic surgery. Institutions involved in research on synthetic calcium phosphate bioceramic materials are Department of Oral and Maxillofacial Surgery, Institute of Anatomy and Anthropolgy, Department of Pathology, Department of Biology and Microbiology, Experimental Animal Laboratory at Riga Stradiņš University, Rudolfs Cimdins Biomaterials Innovation and Development Centre at Riga Technical University, Laboratory of Biodosimetry and Bioanalythical Methods, Faculty of Biology at the University of Latvia, Department of Orthopedic Surgery at Riga 2nd Hospital.

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2. The use of Hap in oral and maxillofacial surgery/D. Paegle, M. Pilmane, G. Salms, I. Salma, R. Cimdins, L. Berzina//13th European Conference on Biomaterials, Goteborg, Sweden, 4–7 September.
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12. G. Salms, I. Salma, A. Skagers, J. Locs/3D cone beam radiodensitometry in evaluation of hydroxyapatite (HAP)/tissue hybrid after maxillary sinus floor elevation//Advanced Materials Research Vol. 222 (2011) pp 251–254, Trans Tech Publications, Switzerland doi:10.4028/www.scientific.net/AMR.222.251.

BIOCERAMICS IN ORTHOGNATHIC SURGERY

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Orthognathic surgery is a well-established surgical treatment method for patients with skeletal bite discrepancies. Sometimes during extensive jaw elongation procedures an undesirable sand-watch effect is achieved in the site of osteotomy and the dip in the jaw contour can be visible if the patient has thin soft tissue. Also, asymmetric jaws not always can be corrected to full symmetry by changing the jaw position without altering the shape of the jaw itself. For many years the autogenous bone grafts had been used to augment osteotomy sites and for contourplasty in order to overcome the before mentioned aesthetic drawbacks. However, the resorbtion of various rates made this option unpredictable and not feasible for the use in the aesthetic field.

The aim of this study was to investigate the volumetric stability of particulated hydroxyapatite and biphasic ceramic (HAP/TCP 90/10) in the sites of osteotomy after orthognathic surgery and subperiostally placed during contourplasty.

MATERIALS AND METHODS

Thirteen patients scheduled for orthognathic surgery and controurplasty were enrolled into retrospective clinical trial. HAP/TCP mixed with a small amount of collagen powder was demoistured and set under infrared light and implanted into the sites of osteotomy after perorming the osteosynthesis or for onlay recontouring of jaws and zygomatic bones. CBCT scans were performed before the operation, one week after the operation, 4 and 12 months after the operation. The mean follow-up of the patients was 11 months. Volumetric change in graft volume was assessed by means of 3D voxel superimposition and object subtraction in Simplant 13.0 OMS software (Materialise, Belgium). Calibration was performed on two data sets with the interval of 2 weeks by the same examiner to determine the intra-examiner accuracy and reproducibility of measurements.

RESULTS

The intra-examiner reproducibility for 3D superimposition of CBCT scans was very high (0.95). The changes in graft volume as determined by CBCT superimposition and subtraction were minimal (<5%) in a long term at 12 months and <10% in a short term during the first 4 months after the surgery. The redistribution of the graft rather than loss of the volume has been noticed in the short-term follow up in CBCT. No inflammatory complications had been reported.

CONCLUSIONS

Particulate HAP/TCP with biphasic ceramic (HAP/TCP 90/10) is a predictable material for filling the closed defect sites during orthognathic surgery and for contourplasty due to excellent volume stability.

IN VITRO EVALUATION OF BIOMATERIALS SYNTHESIZED IN RIGA TECHNICAL UNIVERSITY

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In vitro evaluation of biomaterials is a very important aspect of testing that guarantees the safety and effectiveness of its further in vivo and clinical application. The cytotoxicity test and proliferation assay is a biological screening method, known to be an important and frequently used method to appreciate biocompatibility of biomaterials. The aim of this work was to investigate cytotoxicity and biocompatibility of bioceramics and polyvinyl alcohol (PVA) biogels, synthesized in Riga Technical University. These biomaterials are currently used for the bone and dental implantation and wound care.

Cell culture techniques have tended to be used in biomaterial research as a screening method prior to embarking on specific in vivo studies. Our method of screening was based on the use of fluorescent cell lines that has allowed improving visualization of cell spreading on the surface of samples and cells morphology. For this purpose genetically modified human osteoblast-like cells MG-63 (from ATCC collection), producing *Green Fluorescent Protein* (GFP), and GFP–expressed cell lines PT-67 (mouse embrional fibroblast, from Clontech), were developed. Cell lines were cultured in supplemented DMEM medium in the wells of 24-well plate with/without biomaterials. Cytotoxic effect was appreciated in two basic colorimetric assays – MTT [1] and Crystal violet [2] staining of cells cultivated for 24 to 72 hours. The dye absorbance was measured using microplate reader. The absorbance data and software PRISM were used for the calculation of the percentage of alive cells on the tested surface. All data were the mean of three independent experiments. The results of biocompatibility investigations regarding the cytotoxicity of biomaterials were evaluated as a percent of alive cell covered sample surface in 24–72h if compared to the control – well of culture plate, which was taken as 100%. Inverted fluorescent microscopy allowed determining topography of cell growth depending on sample structure and/or composition.

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BONE DEFECT MANAGEMENT IN ORTHOPAEDIC SURGERY WITH APPLICATION OF BIPHASIC CALCIUM PHOSPHATE

S. Petronis[1,2], J. Petronis[2], V. Zalite[3], J. Locs[3], A. Skagers[1], M. Pilmane[1]

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INTRODUCTION

Numerous studies have already revealed the superior osteoinduction of implanted biphasic calcium phosphate (BCP) bioceramics consisting of hydroxyapatite (Hap) and β-tricalcium phosphate (TCP) over pure HAp [1]. In the current study the BCP ceramic granules with HAp/β-TCP ratio of 90/10 were selected as appropriate. The study objective is to evaluate the first results of BCP ceramic granules with HAp/β-TCP ratio of 90/10 as bone substitute for reconstruction of the bone defects in orthopedic surgery.

MATERIALS AND METHODS

Preparation of BCP ceramic granules

Calcium deficient hydroxyapatite (CDHAp) was synthesized by aqueous precipitation technique, where calcium hydroxide and phosphoric acid was used as raw materials for the reaction $Ca(OH)_2 + H_3PO_4 \rightarrow Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} + H_2O$. The filtered precipitates were formed into granules, dried and sintered at 1150∘C for 2 hours. The sintered granules were sieved using vibrational sieves to gain the granular fraction in sizes from 1 to 1.4 mm.

Clinical cases

This study is a retrospective evaluation of twelve randomly chosen clinical cases of patients who had undergone surgeries with BCP implantation. The follow up period range from 2 to 8 months, average 4.5 months.

Three patients were male and nine female. Age ranged from 22 years to 84 years with mean age 62.2 years.

Bone defects were classified according to the etiology – orthopedic (5 patients), traumatic (4 patients) and due to osteomyelitis (3 patients). Regarding morphology, the defects were divided into cavity bone defects and segmental bone defects that were measured on the digital x-ray images.

Clinical and radiographic evaluation

Clinical (functional, scored 0 to 3) and radiographic (integration of the biomaterial and callus formation, scored 0 to 3) assessments served as the basis for the result classification.

The final results represent the sum of both parameter scores: 0–2 poor results, 3 moderate results, 4–5 good results and 6-excellent results. The classification and evaluation method is similar to the one in recent publications [2].

RESULTS

Clinical results

Combined scores were calculated in each group: the cause and morphology of defect. Our overall results showed that in short term 7 out of 12 patients had good results and the other 5 patients had moderate results.

The size of defects ranged from 1 cm to 32 cm, average 6.42 cm, median 3 cm.

The amount of bioceramics used to fill bone defects on average was 22.5 grams (range 5 g to 60 g).

Comparing patients with a different cause of bone defects we revealed that the best results are after elective orthopedic procedures with good results in four cases out of five. Trauma patients had good results in two cases out of four. Among three cases with osteomyelitis one was with good and two with moderate results.

Regarding the size of the bone defect we saw better results for patients with bone defect less than 3 cm. In this group five out of seven had good results. We also should take into account that the patients with bigger bone defects had more invasive surgery that influences the overall result.

CONCLUSION

The best results were obtained from patients after elective orthopedic procedures and patients with bone defects less than 3 cm.

Our bioceramics is a good alternative to fill small and large bone defects both in cortical and cancellous bone areas and could be a good alternative for bone defect reconstruction.

The first results are positive and we will continue the follow-up of these patients to collect late clinical data of bone defect healing and biomaterial osseointegration.

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MORPHOLOGICAL EVALUATION OF BONE AFTER IMPLANTATION OF HYDROXYAPATITE AND BIPHASIC CERAMIC BONE SUBSTITUTES

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In clinical practice different bone substitute biomaterials are used for maxillary sinus floor augmentation. Due to biocompatibility and bioactivity hydroxyapatite (HAP) and biphasic ceramic are frequently used as bone substitute biomaterials for jaw bone augmentation. Data in the literature on tissue and cell responses to the bioceramic materials are different and sometimes controversial.

The aim of our study was to evaluate bone tissue response to bioceramic bone substitutes – hydroxyapatite (HAP) and biphasic ceramic (HAP/TCP 60/40) in animal experiments.

MATERIALS AND METHODS

Calcium phosphates were synthesized by aqueous precipitation technique. The filtered precipitates were formed into granules, dried and sintered at 1150∘C for 2 hours.

Intraosal implantation of bioceramic granules in the size range from 0.5 to 1 mm was performed in a rabbit jaw. A bone with biomaterial was obtained after three months using 3mm trephine drill. Tissues were proceeded for routine histological examination (haematoxylin and eosin) and the detection of bone morphogenic protein (BMP2) and osteocalcin (OC).

RESULTS

HAP granules showed better integration in bone tissue with remarkable formation of new bone and pronounced expression of OC and BMP2.

ACKNOWLEDGMENT

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EVALUATION OF SINUS-LIFT ENFORCED WITH BIOMATERIALS BY CONE-BEAM COMPUTED TOMOGRAPHY

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OBJECTIVE

An enforcement of atrophic maxilla with biomaterials during sinus-lift surgery improves mineralization of natural bone. Radiological densitometric analysis can prove it. Within the study a density of bone was investigated and findings were compared with similar measurements of bone around the implants where no biomaterials were used.

MATERIALS AND METHODS

The study group included 22 patients at the age from 32 to 68 years (the mean age was 48.8 years), out of them 11 were females and 11 were males. There was examined an area around 48 implants enforced with biomaterials by using densitometric analysis; 25 implants (52%) were inserted into females and 23 implants (48%) were inserted into males.

The control group included 16 implants which were inserted into maxillary alveolar bone.

The density of bone around the implants was measured using Hounsfield units (HU). The measurements were performed according to a standardized pattern.

RESULTS

Densitometric measurements were higher in the study group than in the control group in all points. The measurements in HU were as follows: buccally-caudally – 1018.7/891, apically – 766.9/570.1, palatinally-caudally – 800.6/702.7. The measurements buccaly-cranially and palatinally–cranially were statistically significantly higher in the study group ($p<0.005$). The bone was more dense buccaly than palatinally in both groups.

There were statistically significant differences in all measured fields between females and males in the study group. The density in all measured areas was higher in females with exception of buccally – cranially.

CONCLUSION

Biomaterials provided an excellent integration of implants, and optical density around the implants with the enforced bone was higher. The density was lower in the males.

ANALYSIS METHODS OF ANTIBACTERIAL EFFICIENCY OF RETAINERS IMPREGNATED WITH ANTIBIOTICS

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OBJECTIVES

The use of biomaterial implants in medicine is becoming ever more popular, and on many occasions the use of biomaterial implants becomes a lifesaving procedure. A primary obstacle to a wider use of biomaterial implants is their risk of infection and unsuccessful tissue integration with the surface of biomaterials. Various methods, analysis and tests are used in implant research in order to evaluate biomaterial characteristics, compliance with tissues and interaction with bacteria. The objective of this study is to summarise information available on the primary and the most important methods used in studies on biomaterial retainers that have been impregnated with antibiotic substances.

METHODS

Summarisation of available scientific literature from medical databases. An in-depth analysis of scientific literature.

RESULTS

Modification of the Kirby-Bauer test method is used in order to determine the antibacterial activity of biomaterials impregnated with antibiotic substances, evaluating the sterile area around the biomaterial disk. Similar methods are used to determine medication secretion *in vitro*. Sonification and spectrophotometry methods are used in order to determine whether biomaterials are impregnated with antibiotics, and what the concentration of antibiotics is. Sonification is also used to evaluate bacterial adhesion and colonisation abilities on the surface of biomaterials. Biomaterial surface characteristics, porousness, bacterial adhesion and colonisation abilities, as well as biofilm formation and features, are determined with the help of scanning electron microscopy. Depending on the retainer, thermogravimetric analysis may be used to measure the retainer presence on biomaterials.

CONCLUSIONS

Analysis of scientific literature proves that in the studies on biomaterial implants a wide array of methods, analysis and tests are used. Depending on the needs and objectives of the study, methods are chosen which may confirm or deny tasks set out in the study. In our future studies we will use a modification of the Kirby-Bauer test method in order to determine antibacterial sensitivity. Additionally, we will use sonification, spectrophotometry and scanning electron microscopy methods in order to determine the concentration of antibiotics, characteristics of biomaterial surfaces, bacterial adhesion and colonisation abilities.

MORPHOGENESIS AROUND HAP BIOCERAMIC IMPLANTS LOADED WITH AUTHOLOGOUS MESENCHYMAL CELLS AFTER IMPLANTATION IN SUBCUTANEOUS TISSUE OF RABBITS

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We used synthetic porous HAp ceramics with open porosity 61% produced in Rudolfs Cimdins Biomaterials Innovation and Development Centre. Samples as tablets and granules have a macroporous structure with the pore size 50–450 μm. All samples were sintered at 1150°C for 2 hours. Purity of hydroxyapatite phase was investigated using X-ray diffractometry and Fourier transforms infrared spectroscopy. The first step was taking of Dormicum and Ketamine piece of bone from iliac crest under general anesthesia in 10 Californian rabbits, the permission granted by the Latvian Food and Veterinary Service. Bone marrow and bone samples from the rabbits were collected in tubes (Sarstedt) with physiological solution and heparin. The samples were washed with PBS and treated with Collagenase type XI for 2–5 h at 37. The cell pellet was seeded in tissue culture flasks in DMEM/20%FBS. HAp tablets were soaked in 100%FBS for 2 h. Approximately 100 000 cells were applied on the top surface of HAp tablets and DMEM/10%FBS was added up to the surface of HAp tablet. The samples were incubated at 37°C, 5% CO_2 for one day. The samples were washed with PBS twice before implantation. Under local anesthesia with 1% Lidocaine and 1 mg Dormicum intramusculary implants loaded with cells on one side and pure Hap on the other side in subcutaneous tissue of the back were inserted. The samples were taken out after three months, histologic specimens stained with Hem/eoz and Masson trichrome in a light microscope were evaluated.

Two layer capsule formation was observed around HAp implants loaded with cells more intensively as in HAp alone samples. The inner part of the formed capsule consisted predominantly of mononuclear cells/plasmocytes and macrophages cells without inflammatory cells. The external layer of the capsule contained mostly connective tissue with separate microvascularised in-grows through the internal capsule into HAp tablet. An active formation of collagen was observed, whereas no signs of inflammation were detected. The two layer capsule around synthetic HAp granules and tablets loaded with autologous cells is similar to the periosteum structure and might serve as a morphologic structure of bone induction capacity of bioceramics loaded with cells.

ACKNOWLEDGEMENT

This work was supported by the Latvian State Research Program activity 4.5 “New materials and technologies for evaluation and substitution of biological tissue”.

October 19th, 2012
POSTER PRESENTATIONS

TIME-DEPENDING TISSUE CHANGES AFTER IMPLANTATION OF BIOMATERIALS COVERED BY STEM CELLS IN SUBCUTIS AND SUBPERIOSTALY OF EXPERIMENTAL ANIMALS

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INTRODUCTION

Mesenchymal stem cells (MSC) influence the tissue regeneration. Still there are unclear questions about the MSC/biomaterial raised tissue changes around the implant. Our aim was detection of tissue changes in subcutis and bone of experimental animals after seeded by MSC biomaterial.

MATERIAL AND METHODS

The material was obtained from 6 rabbit spine subcutis and jaw bone 5.5 months after the hydroxyapatite/tricalciumphosphate (Hap/TCP) granuli seeded by MSC implantation. The MSC were obtained from the same rabbit. The right side of each animal was the implantation place, but the left side was a control. Tissues were preceded for detection of TNFα, NFkBp105, Hsp70, FGF1R, VEGF, defensing 2 beta, MMP2 and apoptosis.

RESULTS

Results demonstrated perivascular inflammation only in controls. Numerous connective tissue cells of MSC/biomaterial side showed NFkBp105 while this factor decreased in controls. The number of Hsp70-cells was smaller in the experimental side. Apoptosis affected more the experimental side connective tissue and blood vessels. TNFα was absent, but the number of FGF1R immunoreactive structures was similar in controls and around MSC/biomaterial. The number of VEGF positive endothelial cells varied in MSC/biomaterial side, but showed stable numerous numbers in controls.

CONCLUSION

Implantation of Hap/TCP granuli seeded by 3 mg MSC after 5.5 months implantation decreases soft tissue apoptosis and degeneration, it does not affect the expression of antimicrobial proteins, but increases degeneration of osteocytes (possibly with stimulation of faster reabsorbing of the implant).

HUMAN GINGIVAL FIBROBLAST BEHAVIOR ON MODIFIED TITANIUM SURFACES

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BACKGROUND AND AIM

Quality differentiation, proliferation as well as adhesion of fibroblasts to dental materials, such as titanium, are of critical importance to provide efficient soft tissue 'seal'. The aim of the study was to test differentiation, proliferation and adhesion properties of human gingival fibroblasts on differently modified titanium surfaces.

MATERIALS AND METHODS

Six types of titanium surfaces were fabricated using laser beam. Fibroblast cells were extracted and cultivated from human gingival tissues obtained from a patient undergoing gingivectomy procedure. Cell proliferation and cytotoxicity was analyzed by MTT assay. Morphological evidence of apoptosis was obtained using acridine orange-ethidium bromide (AO/EB) staining. In addition, cellular surface specific antigens were detected using flow cytometry to assess cell differentiation. Distribution of focal adhesions was visualized by immunofluorescence staining. SEM analysis was used to visualize cell proliferation on different titanium surfaces.

RESULTS

Results indicated that human gingival cell adhesion at early stages is similar on all tested surfaces. Cell proliferation speed tended to be similar on all surfaces, but slower on the control surface. Focal adhesions were twice as high in all groups, except the control group. The highest focal adhesion activity was observed on sand-blasted surfaces. Human gingival cells appeared to follow the direction of small irregularities on tested surfaces.

CONCLUSION

Within the limitations of this study it may be suggested that adhesion can be controlled through appropriate biomaterial nature and design such as surface roughness. It was observed that laser modified grit surface showed better adhesion qualities compared to other tested surfaces.

EXPRESSION OF BONE REGENERATION PROTEINS IN RABBIT BONE TISSUE AFTER THE IMPLANTATION OF VARIOUS BIOCERAMIC MATERIALS

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INTRODUCTION

Detection of bone morphogenetic protein2/4 (BMP2/4), bone regeneration protein osteoprotegerin (OPG), bone substance proteins osteopontin (OP) and osteocalcin (OC) has an essential role in the evaluation process of biocompatibility.

The aim of our study was to assess the difference among the expression of BMP2/4, OPG, OP and OC in a rabbit bone tissue three months after the implantation of various bioceramic materials.

MATERIAL AND METHODS

Biomaterials containing hydroxyapatite, calcium phosphate and polimethylmethacrylate produced in Riga Technical University and commercial biomaterial were implanted in the bone tissue of rabbit's lower jaw and tibial bone. Three months later the euthanasia of rabbits was performed and tissue blocks were taken out and were prepared for immunohistochemical detection of BMP2/4, OPG, OP and OC.

RESULTS

Signs of new bone developing zones and bone-implant contact were evaluated in experimental tissue with such biomaterials as pure hydroxyapatite (HAp), HAp covered by polycaprolactone (PCL), commercial polymethylmethacrylate (PMMA) bone cement and unburned HAp granules. The expression of BMP2/4, OPG, OP and OC positive osteocytes in the experimental tissue was variable, but in bone tissue with HAp covered by PCL it was more pronounced.

CONCLUSION

New bone developing zones and signs of bone-implant contact and marked expression of BMP2/4, OPG, OP and OC in experimental tissue with HAp covered by PCL feasibly indicate that this material is more osteoinductive than pure HAp used in our study.

AUTOLOGOUS FIBRIN/BIOCERAMIC SCAFFOLD FOR BONE TISSUE ENGINEERING

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The physical, chemical and biological properties of both biphasic calcium phosphate (BCP) bioceramics and fibrin scaffolds may be cumulated for preparing bone substitutes. The aim of the experimental study was to evaluate histological response on implantation of synthetic biphasic HAp/TCP bioceramic granules mixed with autologous plasma derived fibrin scaffold in subcutaneous tissue of rabbits.

MATERIALS AND METHODS

Biphasic ceramic granules with HAp/b-TCP ratio of 30/70 and in sizes from 0.5 to 1.0 mm were selected for in vivo experiments. For the preparation of the bioceramic and plasma scaffold composites, antifibrinolytic agent tranexamic acid is dissolved in the autologous rabbit plasma and then calcium gluconate and 0.5g BCP ceramic granules are added. Six New Zealand male rabbits were used for this morphological study. A 2 cm incision was made on the right side of lumbar area and 0.5g Hap/TCP granules with fibrin scaffold were implanted subcutaneously. 0.5g Hap/TCP granules without fibrin scaffold were implanted on the control side. Blocks of soft tissue from experimental and control side were prepared for detection of apoptosis using the TUNEL method. Routine histological method – staining with hematoxilin and eosin – was used for obtaining a review picture.

RESULTS

Six weeks after the implantation of plasma derived fibrin scaffold with BCP bioceramic granules routine histological examination showed an increased number of cells, mainly plasmatic and gigantic cells, also lymphocytes and eosinophils and increased formation of fibrous tissue capsule compared with the control side. BCP bioceramics with autologous plasma derived fibrin scaffold initiate pronounced angiogenesis around the implant.

CONCLUSIONS

The results allow us conclude that plasma derived fibrin scaffold activates encapsulation of BCP bioceramic in soft tissue environment. This phenomenon may serve as a possibility for biological retention of drugs, growth factors and/or cells.

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IN VITRO AND IN VIVO STUDY OF BACTERIAL ADHESION AND COLONISATION ON DIFFERENT BIOMATERIALS

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INTRODUCTION

The aim of the study was to explore the ability of microorganisms to adhere on originally synthesised biomaterials surface in order to evaluate the microbial contamination risk of these biomaterials and its impact on the surrounding tissue.

MATERIALS AND METHODS

Biomaterial samples:

- Bioceramics
- Hap/TiO2
- Hap/TCP
- Hap/Ag

In vitro study: Samples were cultivated in 1 ml *Ps.aeruginosa* ATCC 27853, *S.epidermidis* ATCC 12228 bacterial suspensions with a concentration of 10, 10^2 and 10^3 CFU/ml (colony forming units). For the evaluation of adhesion we used scanning electron microscope (SEM) as well as sonication and culture method for the determination of colony numbers.

In vivo study: For the preparation of pure cultures we used the same bacterial suspensions in 1 ml volume of TSB concentration of 10^2 and 10^3 CFU/ml. Samples were cultivated at 37°C for 2h to determine adhesion rates and ensure adhesion of the bacteria. Then contaminated biomaterial samples were implanted in interscapular area of chinchilla rabbits for 2 and 4 weeks. The biomaterial was removed and, using plate count and sonification method the bacterial colonisation on the surface of the biomaterial was determined, however, we prepared preparations from the surrounding tissues, staining in haematoxylin-eosin and using immunohistochemistry methods thus determining TNF-A,-B defensin-2 and Il-10.

RESULTS

Bioceramics: Biomaterial samples contaminated with *S.epidermidis* showed a low degree of adhesion and colonisation.

The most intense inflammatory reaction (INF-A and Il-10) was observed around the *Ps.aeruginosa* contaminated biomaterials. Indicators of inflammation practically did not differ for the 2 and 4 week implants of biomaterials. We observed some defensin-containing cells in tissue surrounding biomaterials after *Ps.aeruginosa* infection, while in other biomaterial contamination cases such cell amount were much more moderate.

Hap/TiO2: Results of the experimental data show that the least microorganism adhesion is observed on composites HAp/50%TiO_2 and HAp/80%TiO_2 at 1200°C.

Hap/TCP: The adhesion process of Ps.aeruginosa began at 10 CFU/ml of incubated samples and finally demonstrated around 20 times greater intensity than the adhesion of S.epidermidis, incubated in 10^3 CFU/ml.

Hap/Ag: The adhesion of *S.epidermidis* began at 10^2 CFU/ml of incubated samples with different Ag additives, but *Ps.aeruginosa* adhesion occurred at 10^3 CFU/ml of incubated samples.

CONCLUSIONS

Bioceramics: The study showed that *Ps.aeruginosa* compared with *S.epidermidis* more intensively colonised biomaterials in the *in vivo* study. *Ps.aeruginosa* infection tends to cause depletion of B-defensin 2 production in tissues.

Hap/TiO2: Microorganisms adhere considerably less on denser materials, since at 1200°C the microstructure of composite ceramics is not as porous.

Hap/TCP: The adhesion and colonization intensity of *Ps.aeruginosa* on the surface of the all samples was much higher than that of *S.epidermidis*, as well as the adhesion and colonization intensity of both bacteria was higher for the ceramics containing larger TCP amount.

Hap/Ag: Bacterial adhesion and colonisation is best decreased by biomaterials of 0.5 g Ag additives processed at 1150°C

ACKNOWLEDGMENT

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October 20th, 2012
ORAL PRESENTATIONS

DETERMING A CLINICAL RELEVANT BONE ENGINEERING METHOD. AN ALL IN ONE'' STUDY IN NUDE MICE

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INTRODUCTION

Craniofacial bone reconstruction is still a challenge in irradiated, traumatized or contaminated tissues. Autologous bone graft (ABG) exhibits some risks and side effects. Bone substitutes like Biphasic Calcium Phosphate (BCP) as calcium phosphate ceramics cannot provide a sufficient bone healing. In such conditions, but also in ectopic area in animal, osteoprogenitors are essential for providing osteoinductive properties to the BCP [1]. Numerous bone tissue engineering (BTE) strategies have been described in the past but they didn't completely address the clinical issues with respect to efficacy, simplicity, and cost [2]. In an attempt to determine the most clinically relevant strategy, we sought to compare some of the main successful BTE procedures in an "all in one" study in nude mice ectopic subcutaneous site.

EXPERIMENTAL METHODS

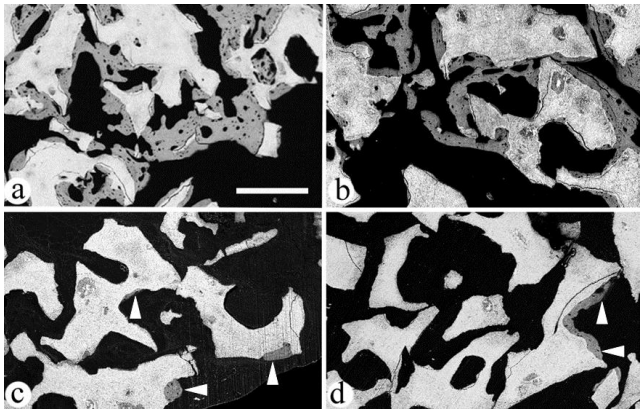
The biomaterials used was BCP granules (MBCP™ 0,5-1mm size, 60% hydroxyapatite – 40% beta-tricalcium phosphate). Thirty adult S/SOPF Swiss nude mice were divided in 10 groups. They were subcutaneously implanted (2 implants per animal) with BCP combined or not with Wistar rat bone marrow mesenchymal stem cells (MSC). **GROUP A** (positive control): BCP + ABG, **GROUP B** (negative control): BCP alone, **GROUP C**: BCP + unfractionated total bone marrow (TBM), **GROUP D & E**: BCP + 90 000 MSC/cm² osteogenically committed (D) or not (E) by coculture on BCP, **GROUP F & G**: BCP + 45 000 MSC/cm² osteogenically committed (F) or not (G) by coculture on BCP, **GROUP H & I:** BCP + 90 000 MSC/cm² osteogenically committed (H) or not (I) by culture on plastic and extemporaneously loaded on BCP, **GROUP J**: Highly confluent committed MSC (cell sheets). Mice were sacrificed at 8 weeks. The bone formation was assessed by scanning electronic microscopy (SEM), and histology.

RESULTS AND DISCUSSION

Only 4 groups demonstrated an ectopic bone formation (table 1 and figure 1). The highest rate (6/6) of bone formation was seen in group A (positive control) and group C (BCP+TBM). A lower rate (3/6) was seen in group D (BCP + 90 000 committed MSC by coculture) and group H (BCP + extemporaneously loaded committed MSC). No bone formation was detected in the remaining groups.

Group	A	B	C	D	E	F	G	H	I	J
Bone formation	+++	-	+++	+	-	-	-	+	-	-
Nb of positive implants	6/6	0/6	6/6	3/6	0/6	0/6	0/6	3/6	0/6	0/6

“Table 1: Number of samples exhibiting an ectopic bone formation in each tested group. Bone formation rate was scored +++: high; + slight ; - not detected.“



”Figure 1: SEM observation of newly formed bone a: Group A. b: Group C. c: Group D. d: Group H.” Arrowhead indicates newly formed bone, bar =500 µm.

CONCLUSION

This study clearly demonstrated the positive effect of associating TBM to a BCP for ectopic bone formation. Given its usability and efficacy, a total bone marrow-based strategy could be considered as a relevant alternative to ABG but also to current BTE procedures that use committed or not MSC

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NEW APPROACH FOR DEVELOPMENT OF OSTEOPLASTIC MATERIALS

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INTRODUCTION

The problem of bone defects repair is highly relevant to surgical practice, but it has not been solved yet. The current trends in developing osteoplastic materials include creation of products with active components such as the growth factors and cells. However, these approaches have several disadvantages that limit their use in clinical practice.

MATERIALS AND METHODS

In this context we aim to develop a fundamentally new class of osteoplastic materials, containing the nucleic acids as an innovative active component. We combined a scaffold (collagen/hydroxyapatite) with DNA-plasmid containing VEGF/GFP genes. The choice of DNA-plasmid with VEGF for the creation of the first exponent of new class osteoplastic materials was caused by the fact that angiogenesis is a critical factor for reparative osteogenesis. The produced «gene therapeutic osteoplastic material» was investigated in vitro (cultured with MMSC and endotheliocytes on Matrigel) and in vivo (transplanted into cranial bone defects of rabbits).

RESULTS

High angiogenic potential of gene therapeutic osteoplastic material was detected in vitro. We found that in vitro plasmids of gene-therapeutic osteoplastic material leave the structure of a scaffold and transfer into MMSC where the genes of plasmids are expressed. Edotheliocytes form capillary-like structures on Matrigel. The results of experiment in vivo will be reported by our colleagues.

CONCLUSION

We consider that gene-therapy osteoplastic material is the most promising for clinical practice due to its high efficiency and lower costs in comparison with cellular products.

CALCIUM PHOSPHATE BIOCERAMICS FOR REINFORCEMENT OF BONE

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This work was aimed at the development of biocompatible materials based on calcium phosphate and intended for both the replacement of damaged bone tissue and its regeneration. Specific features of the synthesis of calcium phosphate powders and the fabrication of ceramic implants and bone cements are investigated. Advances in the development of porous scaffolds from biodegradable and osteoconductive calcium phosphates that form the basis of bone tissue engineering are considered.

ACKNOWLEDGMENT

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THE ROLE OF SILICON SUBSTITUTION ON B-TRICALCIUM PHOSPHATE CERAMIC

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INTRODUCTION

The use of bone regeneration procedures is becoming a daily practice in dentistry and clinicians are demanding higher efficient bone substitutes. Beta-tricalcium phosphate is a transductive bone substitute that is widely used in maxillofacial and orthopedic field. We believe that ionic substitution of phosphorous by silicon will improve and widen the therapeutic efficiency of such ceramic.

EXPERIMENTAL METHODS

B-TCP was prepared by the calcinations of brushite and calcium carbonate mixture of a molar ratio of 1.5. Ionic-doped ceramics were prepared by substituting brushite with SiO_2 at different molar ratios. Structural characterization was performed by X-ray diffraction and Scanning electron microscopy (SEM). Porosity and specific surface area (SSA) were also determined. Ceramics were cultured with osteoblast cell line MG 64 and the in vivo behavior by granulate implantation in critical bone defect in rabbit calvaria.

RESULTS AND DISCUSSION

Si-doped ceramics are mainly composed of β-TCP and silicocarnotite ($Ca_5(PO_4)_2SiO_4$) (see Fig.1). Furthermore, porosity and surface area was increased by silicon substitution.

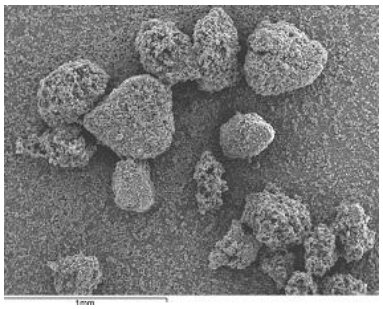

Figure 1. SEM micrograph of Si-doped β-TCP

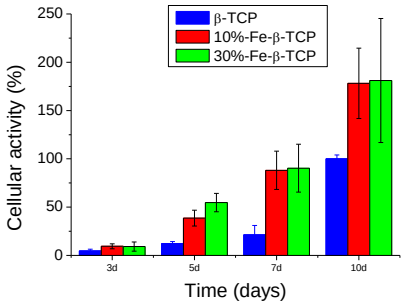

Figure 2. Silicon doping and MG 63 response to β-TCP

The silicon substitution was proved to improve significantly the osteoblast proliferation and activity which was manifested in improved bone regeneration achieved by filling the created bone defects. This was testified by the histological analysis of bone samples harvested after 8 and 12 weeks of implantation (Fig 2).

CONCLUSION

Ionic substitution is an efficient tool to generate materials with gradient properties that help toward the development of intelligent biomaterials.

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ADVANCED TITANIUM ALLOYS AND FOAMS FOR ORTHOPEDIC APPLICATION

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INTEGRATION OF CALCIUM PHOSPHATE BIOCERAMICS IN MAXILLARY SINUS FLOOR

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Bone augmentation in the posterior part of maxilla is a daily routine procedure with high success rate, however, the bone quality before and after the graft placement is frequently underestimated.

The bone quality and quantity is the most important factor for successful grafting and dental implant. The aim of this experimental study was to evaluate the bone quality and quantity before implantation and after bone regeneration by using bone radiological and morphological investigation.

MATERIALS AND METHODS

Trephine biopsies were taken from twenty patients before and after sinus lift operations and investigated morphologically and immunohistochemically. Bone radiodensitometry was analyzed for the same patient group before and after implantation of the bone substitute material.

RESULTS

The bone volume was diminished before implantation; it was proved radiologically and clinically, but the radiodensity data were variable. The bone radiodensity was sligtly higher in the palatal part of residual bone after implantation than before implantation. Radiodensity was slightly higher in the bone hybrid part bucally comparing with hybrid palatally. All patients showed a pronounced expression of osteocalcin (OC) and osteopontin (OP) which are bone mineralization and regeneration markers. Analyzing apoptosis, TGF beta, BMP 2/4, MMP 9 high variability were observed from negative to marked positive reactions.

CONCLUSION

Bone/biomaterial hybrid density was elevated after biomaterial implantation into maxillary sinus. Marked expression of osteopontin showed the patient bone remodelling and mineralization potential after bone grafting. Increased expression of osteoprotegerin after bone grafting indicated the increased activity of bone cells (osteoblasts). Commonly, bone tissue reactogenity is variable and strongly individual that does not always correlate with the radiological findings.

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ENGINEERING OF BIOCERAMICS IN LATVIA: TECHNOLOGIES AND PRECLINICAL ASSESSMENT

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Research of bioceramics in Latvia started in the mid-1990s after the first commercial bioceramic implants reached the newly opened Latvian market and began to be used in practice.

The necessity of this research was caused by widely varying properties of available commercial implants and subsequent difficulties to determine the clinical result of implantation. Our group began to produce our own bioceramics, but using commercial starting materials. Subsequently, we also began to synthesize starting materials for calcium phosphate ceramics with predictable phase composition and other properties.

Long term clinical trials of partly or fully sintered biphasic calcium phosphate bioceramics with different porosity gave excellent clinical results – the bone fully recovered its initial volume and mechanical properties of the resulting bone were greatly improved.

In 2006 Riga Biomaterials Innovation and Development Centre at Riga Technical University was established, using the funds of PHARE 2003. This year it was named Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre after its founder. After the establishment of the Centre research in the biomaterial field was intensified and broadened. The research is now truly multidisciplinary – the staff includes chemists, materials scientists, medical specialists and microbiologists. On the basis of in-depth research of calcium phosphates, calcium phosphate cements, bioceramic matrices for controlled drug delivery and bioceramics with controlled micro- and macroporosity are developed. In vitro and in vivo research of these materials is also conducted.

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October 20th, 2012
POSTER PRESENTATIONS

SYNTHESIS METHODOLOGY AND INVESTIGATION OF CALCIUM PHOSPHATE BIOMATERIALS FOR BONE TISSUE REPLACEMENT IN REGENERATIVE MEDICINE

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The wet chemical precipitation method was chosen for synthesis of calcium phosphates. It is one of the methods to obtain calcium phosphates that demonstrate both complexity of the method from the point of view of physical chemistry and variability of product properties by controlling synthesis parameters. The method is based on neutralization of $Ca(OH)_2$ suspension with solution of phosphoric acid:

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O.$$

Contrary to the conventional chemical precipitation route $Ca(OH)_2$ was homogenized with planetary mill. Milling CaO and water in planetary ball mill as the first step of synthesis provides a highly dispersed $Ca(OH)_2$ suspension.

The aim of this work was to study the influence of main processing parameters of wet chemical precipitation synthesis product and to control the morphology, phase and functional group composition and, consequently, the thermal stability and microstructure of calcium phosphate bioceramics after thermal treatment.

Fourier-transform infrared spectroscopy, X-ray diffraction, and Field Emission Scanning Electron Microscopy were used to investigate starting materials of synthesis, synthesized powders and sintered bioceramic samples.

The results showed that it is possible to obtain calcium phosphates with different and reproducible phase compositions after thermal treatment (hydroxyapatite [HAp], β-tricalcium phosphate [β-TCP] and HAp/β-TCP) by modified wet-chemical precipitation route by precise control of composition and structure of calcium containing raw materials and properties of suspension for start of synthesis. Pure, crystalline and highly thermally stable (up to 1300∘C) HAp bioceramics with homogenous microstructure was obtained from powders synthesized at elevated synthesis temperature, stabilizing ending pH 9 and after aging of suspension for 20 h.

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MECHANICALLY STRONG BIOACTIVE CALCIUM PHOSPHATE GLASS AND GLASS-CERAMIC

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Calcium phosphate biomaterials are promising materials for bone tissue replacement; however, often contradictory qualities are required, such as high solubility and mechanical strength. This problem could be solved through the use of composite materials such as glass-ceramics.

The original composition glass-ceramic in the system CaO-Nb_2O_5-P_2O_5-Na_2O was studied. It was found that by controlled crystallization a composite with soluble and practically insoluble phases was produced. In acidic environment soluble $Ca_3(PO_4)_2$, $Ca_2P_2O_7$ can be combined with almost insoluble amorphous or crystalline $Na_4Nb_8P_4O_{32}$. Dissolution of calcium phosphates forms open porosity till 57 vol%. The crystal size and shape can be varied by heating rate, average crystal size is around 0.35–0.66 mm in width and 3–4 mm in length, that is enough for tissue ingrowth after calcium phosphate crystal dissolution.

8N microhardness is higher than in other phosphate glasses and glass-ceramic, reaching ~5.6 GPa. Compression strength reached 117.3 MPa. More crystalline samples have higher mechanical strength.

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EXTRUSION AND THERMAL TREATMENT OF TIO_2 CERAMIC FOR VARIOUS APPLICATIONS

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Titanium dioxide is one of the most important inorganic products in chemical industry. It is widely used in various fields of development of materials – in photocatalysis, biomaterials, self-cleaning elements and in electronics. Many of useful TiO_2 properties arise from various defects in its structure such as nonstoichiometry.

Extrusion is perspective forming technology for obtaining of objects with certain profile. Nowadays it is widely used as forming technology for development of ceramic materials because it is relatively cheap and productive.

After thermal treatment of TiO_2 ceramic at different temperatures and conditions (air or vacuum) it is possible to obtain materials for various applications – for development of electrode materials for water treatment, thermoelectric materials or implant materials.

DEVELOPMENT OF POROUS CALCIUM PHOSPHATE CERAMICS IN RUDOLFS CIMDINS RIGA BIOMATERIALS INNOVATION AND DEVELOPMENT CENTRE

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Calcium phosphate materials are widely used in the medical field as bone grafts and scaffolds, due to biocompatibility and chemical similarity with natural mineral phase of bone. Calcium phosphate ceramics are bioactive and bioresorbable materials. The most popular way to govern bioresorbtion is by changing the molar ratio of calcium and phosphorus, but another possibility is by changing the structure of the material, in this case, porosity. Pore size, pore connectivity and level of porosity can determine resorbtion time of the material. There are developed many methods to obtain porous structure, e.g. sponge impregnation, replication technique, freeze casting, direct foaming etc. The challenge for scientists is to gain material with properties like natural bone.

Development of calcium phosphate biomaterials is one of the main research fields in Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre. Scientists have developed various forms of porous implants and scaffolds, e.g. cylinders, tablets, granules and blocks. The obtained materials are examined with various methods: X-ray diffractometry, Fourier transform infrared spectroscopy, scanning electron microscopy, optical dilatometry and differential thermal analyses. In vitro and in vivo tests are used for studies of cytotoxicity and bioactivity. The above mentioned analyses approve biocompatibility, chemical purity and adequacy of the structure of the developed materials for biomedical application.

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IMPROVEMENT OF CALCIUM PHOSPHATE CEMENT COHESION USING LIQUID PHASE ADDITIVES

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It has been described in scientific literature that the presence of polymeric additives in cement liquid phase can improve cohesion of calcium phosphate cements [1–3]. The influence of liquid phase composition, including water-soluble polymers, on calcium phosphate cement cohesion and setting time has been examined in this work.

Several water soluble polymers (sodium alginate, polyvinyl alcohol, carboxymethyl cellulose sodium salt, hydroxyethyl cellulose) were added to the liquid phase of the cement. The solid phase of the cement was α-tricalcium phosphate. Mixtures of sodium phosphate salts were added to either solid or liquid phase of the cement to ensure setting. The cement cohesion was evaluated both visually and by the weight of particles released from cement surface.

It was found that cement cohesion strongly correlated with the cement setting time. Cements that had the fastest setting time exhibited the best cohesive properties – fewer particles were released from the cement surface. The presence of sodium alginate and carboxymethyl cellulose additives did not decisively improve cohesion. Polyvinyl alcohol improved cement cohesion and also shortened the setting time. Hydroxyethyl cellulose improved cement cohesion if basic phosphate salt mixture was used as a setting agent.

Cohesion was also improved if the mixture of calcium phosphate salts was added to liquid phase instead of solid phase.

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SCALE – UP AND WET PRECIPITATION SYNTHESIS OF CALCIUM PHOSPHATES

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Hydroxyapatite (HAp), tricalcium phosphate (TCP) and their composites – biphasic calcium phosphates (BCP) – are calcium phosphate (CaP) ceramics, one of the most used implant materials in the reconstructive surgery to repair damaged hard tissues. CaP has excellent properties due to bioactivity of HAp and bioresorbability of TCP.

In the last twenty years the main interest is devoted to the CaP synthesis. The progressing requirement for calcium phosphate bioceramics promotes scaling up laboratory synthesis. In this study CaP powders (HAp, TCP, BCP) were prepared by wet chemical precipitation method from calcium oxide and ortophosphoric acid solution, the process can be described by the following reactions equations:

$$CaO + H_2O \rightarrow Ca(OH)_2$$

$$10Ca(OH)_2+6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

The precipitation process is the most reported method for preparing CaP powder. It is a simple, low cost process and suitable for industrial production. Bioactivity of CaP materials depend on many factors during the synthesis procedure, such as the type of precursor reagents, impurities, concentration of reagents, mixing conditions, pH and temperature.

By using the developed method it is possible to obtain 1.0 kg of pure CaP powder **in increased reaction volume** (molar concentration of calcium hydroxide suspension is 0.50 mol/l and phosphoric acid – 2.00 mol/l) compared with laboratory reactor – 0.03 kg CaP. The research revealed that mixing has noticeable influence on phase composition of the precipitation product. Effective mixing promotes formation of HAp phase. Other key factors for successful realization of large scale HAp synthesis are temperature and ortophosphoric acid addition flow rate.

X−ray diffractometry (XRD) was used to analyze phase composition of the obtained bioceramic samples. Fourier-transform infrared spectroscopy (FT-IR) was used to determine the various functional groups in the sintered calcium phosphate samples. Scanning electron microscopy (SEM) was used to characterize the morphology of products.

PREPARATION OF CONTROLLED RELEASE DRUG DELIVERY SYSTEMS BASED ON CALCIUM PHOSPHATES

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Advanced drug delivery is one of the most intensively investigated fields in the last decade, indicating to society's demands for new effectively controlled and site specific drug delivery systems. Musculoskeletal diseases and disorders often demand a drug treatment of specific defect, surgery or injury site. The conventional method of supplying a patient with pharmacologic substances has been either through injection or oral ingestion. Generally speaking, both methods suffer from being very poorly selective, as damage can occur to healthy tissues and organs, different from the intended target. Moreover, high drug doses can be required to achieve the desired effect. An alternative approach is based on the use of implantable delivery tools, able to release the active substance in a controlled way.

Bone is a living tissue which continuously rebuilds its structure and is capable of spontaneous regeneration. Calcium phosphate (especially hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] and β-tricalcium phosphate [$Ca_3(PO_4)_2$]) biomaterials are the most prospective materials for bone tissue replacement and regeneration due to their unique properties – biocompatibility, bioactivity and osteoconductivity. We have prepared bioceramic scaffolds for the site specific lidocaine, gentamicin, doxorubicin and vancomycin delivery ensuring the dual effect – promoting the bone tissue regeneration and supplying the surrounding tissues with certain drugs in a controlled way.

Another approach to preparing controlled release drug delivery systems is encapsulation of active ingredients in a polymer matrix. Microencapsulated drug delivery systems offer numerous advantages, including rate-controlled release of the active ingredient, protection of the encapsulated materials against oxidation and humidity etc. Microencapsulated form of vancomycin have been prepared and incorporated into calcium phosphate bone cements. New researches towards stroncium ranelate and zoledronic acid microencapsulation for the local treatment of osteoporosis have been proposed.

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RECOMBINANT HBCAG PRODUCTION IN *E. COLI*

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Biotechnological aspects in the field of recombinant protein production were analysed in this work which includes the investigation of factors for the production of VLPs formed by recombinant Hepatitis B virus core-antigen HBcAg in inducible bacterial (*E. coli*) expression system. The expression system *E. coli* RB791 IS104-31 used in this study is based on the expression plasmid pQE60 (Qiagen) basis, where the transcription of HBcAg gene is controlled by T5 promoter and two Lac operators.

Biomass yield and VLP production level depending on cultivation conditions such as medium content, carbon source, intensity of aeration, temperature and conditions of induction were investigated in the study.

The maximum level of protein expression in shake-flask experiments was reached at the temperature of 30 ∘C, using peptone-yeast extract culture medium with added phosphates, applying lower aeration, using 0.2% lactose as inductor and 0.2% glucose as the carbon source.

The promoter (*E. coli* RB791 IS104-31) was cultivated in New Brunswick bioreactor (volume 3.5L) in the above described conditions. The optical density 29 (A_{560}) corresponding to the biomass yield of 40 g/L was obtained. The HBc protein expression was twice lower than in the shake-flask experiments.

INVESTIGATION OF SILVER DOPED HYDROXYAPATITE

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Biomaterials based on calcium phosphate ceramics are used as implants in human/animal body due to their excellent biocompatibility. Implantation is associated with bacterial infections that can lead to complications [1]. Silver containing materials have a very broad spectrum of antibacterial activity; therefore silver doped hydroxyapatite can be used in medicine as antibacterial implant material [2]. The aim of this work was to synthesize monophasic and biphasic silver doped hydroxyapatite and evaluate the differences in their physical and antibacterial properties.

Modified wet chemical methods were used to synthesize silver doped hydroxyapatite (HAp/Ag) scaffolds with silver amounts up to 5% weight. CaO or $Ca(NO_3)_2$ were used as raw materials. The dried precipitate was uniaxially pressed and sintered at the temperature range from 900 ∘C to 1200 ∘C. The prepared scaffolds were used for phase composition analysis and inner structure evaluation. Antibacterial properties and their dynamics depending on silver content and phase composition in HAp/Ag were determined.

Characterization studies of XRD showed that the obtained particles are monophasic HAp/Ag by using $Ca(NO_3)_2$ as raw material and biphasic HAp/Ag with combination of silver oxides by using CaO as raw material. Sintering shrinkage analysis of HAp/Ag material demonstrated that monophasic HAp/Ag samples have 200 ∘C higher sintering temperature than pure HAp. It was observed that the sintering temperature depends on silver incorporation in structure and silver concentration in HAp/Ag composites. Samples containing Ag and sintered at 1150 ∘C presented excellent antimicrobial properties compared to HAp/Ag samples sintered at 1000 ∘C temperature and pure HAp samples.

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