

Nantes France

ISACB 6

6th International Symposium Apatite and
Correlative Biomaterials

June 6-7, 2013

Conference in honour of Prof. Racquel LeGeros



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Editorial

This is a unique opportunity for all those involved in science, research and development, technology and clinical application of apatite and related biomaterials to come together with researchers, medical and dental clinicians, veterinary surgeons and allied health disciplines to promote the worldwide advancement of Apatite Science and Technology in Tissue Engineering and Regenerative Medicine.

This specific Interdisciplinary Symposium integrates developed apatite and related experimental research and development and clinical needs. ISACB 5 was organized in 2010 in Australia. Three years after ISACB6 will be in Nantes, a recognized University for Bio ceramics development.

However this year, Professor Racquel Zapanta- LeGeros, one of the pioneers of this symposium, passed out in Nantes, January, 14th. She was in Nantes for preparing the Symposium; she was our friend, our mentor. ISACB6 this year will be dedicated to her.

It will be the aim of the 6th edition of the symposium to bring a single session meeting with invited lectures by international speakers and founders of ISACB. Innovative topics, new biotechnology approaches and stimulating clinical debates will be led by a balance of international and national speakers and the opportunity for industry to contribute.

The meeting will pay particular interest to current needs, expectations, directions and goals for the progress of the development, production and use of apatite, apatite containing composites, apatite with biogenic additives, characterization methods, animal and clinical trials, clinical applications, properties and applications for tissue engineering and regenerative medicine by scientists and clinicians.

In addition to scientific events and the trade exhibit, you can take some reasonable time to visit the historical place of Nantes and most famous beach of Europe (La Baule), and to appreciate the French gastronomy with the Brittany sea food.

Guy Daculsi

Chairman

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ISACB6 was hosted by:

Biomaterials Networks

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Racquel LeGeros Awards Ceremony

The Organizing committee of the International Symposium on Apatite and Correlative Biomaterials (ISACB 6) during 6-8th June, 2013 at Nantes University, France have decided in honour of Racquel LeGeros to create two International awards:

Racquel Legeros Senior Award:

Professor Maria Pau Ginebra (Spain)

Professor Kunio Ischikawa (Japan)

Racquel Legeros young Scientist Award):

Dr. Pascal Borget (France)

Dr. Toshiisa Konishi (Japan)

Dr. Christoffe Drouet (France), honorary award

The awards recognized seniors and younger scientists having largely contributed to the Science of Biomineralization, Calcium phosphate, Bioceramics and participation to the ISACB6 meeting.

Three International Industrial Sponsors, close to Racquel LeGeros and invested in Bioceramics, biomaterials, calcium phosphate manufacturing and distribution have contributed to the ISACB6 awards.

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INVITED LECTURES

Thursday 6 Calcium phosphate and Biotechnologies, 7th PCRD support

“Bone regeneration using mesenchymal stem cell and biomaterials -The Reborne experience 7th PCRD EC”.

P. Layrolle, Invited lecture.

Bone is the most transplanted tissue in human with about 1 million procedures annually in Europe. Autologous bone graft is the gold standard in bone regeneration but it requires a second surgery, is limited in quantity and often associated with complications. Synthetic calcium phosphate biomaterial in association with mesenchymal stem cells is a potent alternative to autologous bone grafting. Starting from a bone marrow aspirate, several hundred millions of mesenchymal stem cells are produced in 3 weeks in culture medium containing human blood platelet lysate. These cells are fixed on calcium phosphate granules and then implanted in subcutis of nude mice where they produced mature bone tissue. The mixture of human mesenchymal stem cells and biomaterial is also effective in bone healing of critical size defects in calvarias and femurs of nude rats. The procedure has also proven efficacy in regenerating diaphysis defects in metatarsi of sheep. The European project REBORNE aims at bone regeneration at the clinical level in non union fractures, treatment of osteonecrosis of the femoral head as well as jaw augmentation prior to dental implants and reconstruction of cleft palates in children. This presentation will give the latest pre clinical results that have led to the authorization by medicinal agencies to translate this regenerative medicine technology to patients.

“Development of gene activated matrices for tissue regeneration in osteoarthritis- The GAMBA experience 7th PCRD EC”, M. Anton, Invited Lecture.

Osteoarthritis is a degenerative disease of the joints that comprises degeneration of cartilage and underlying bone as well as inflammation. Due to unknown etiology treatment is mainly symptomatic ranging from pain reduction and anti-inflammatory drugs to ultimately joint replacement.

The aim of the EU-funded project GAMBA is to develop a Gene Activated Matrix (GAM) concept that allows for spatio-temporal control of growth factor expression involved in cartilage and bone regeneration. GAMs are biomaterial scaffolds comprising gene vectors. Cells growing on or into the matrix will become infected/transfected by the immobilized or released vector and will consequently express the growth factor genes, resulting in local autocrine and paracrine stimulation of a desired differentiation process.

Resorbable bone substitute material (MBCPTM) and a thermo-reversible hyaluronan based hydrogel (HA-gel) are used as scaffolds. Bone marrow-derived mesenchymal stem cells are incorporated into scaffolds together with different gene vectors coding for reporter genes or growth factors involved in cartilage and bone regeneration (BMP-2 and TGF β) and an anti-inflammatory gene (vIL-10). Temporal control of gene expression is achieved using the Tet-on system.

Spatial control of gene expression was achieved by differential loading of gene vectors expressing reporter genes in 3D culture. Temporal control was demonstrated by doxycycline induction of growth factor release in 2D and 3D culture.

Spatial and temporal control using MBCPTM and the HA-gel as scaffolds was achieved using non-viral and adenoviral vectors. Our results indicate that the concept of GAM might be feasible for regulated growth factor expression upon command and demand. Analysis of gene expression induced differentiation of MSC is ongoing.

The scientific and technological objectives of the GAMBA project are complemented with an innovative program of public outreach, actively linking patients and society to the evolvement of this project. GAMBA is funded by 7th Framework Programme of the EU, grant number NMP3-SL-2010-245993.

“Bone Tissue Reactions of HA coated on metal by various techniques

Aoki H., International Apatite Institute Co.

Hydroxyapatite (HA) coating metal for dental implants, hip and knee joints have been used clinically worldwide due to the excellent bone integration. However, the bone tissue reaction and integration of the HA coating metal are largely different in coating techniques including plasma spraying, flame spraying, sputtering, dipping, biomimetic, thermal decomposition, alkaline treatment and others. Because the characterization and property of the coated HA are different by the techniques.

First of all, the thickness of HA coated on metal are very different. The plasma spraying thickness is in range of 35~100 μ m, the flame spraying 10~20 μ m, the dipping or immersing 10 nm~100 μ m, thermal decomposition 1~5 μ m, alkaline treatment and sputtering are approximately 1 μ m. The thickness affects to dissolution or absorption by remodeling in bone tissue. Second, the HA crystal or crystallite sizes are different by the coating techniques. The size affects to bone conduction or induction after implantation. Third, density of HA coating layer is different in each technique. Most of the HA coating layer are porous with 40~60% porosities. The porosity will be relative to infection

and fracture. Fourth, bonding strength between the HA coating layer and metal as substrate is largely different. The strength is becoming smaller as the following: sputtering >alkaline >plasma spraying >flame spraying >dipping=thermal decomposition=biomimetic.

These differences in characterization and properties of HA coated on metal affect to operation procedures and clinical results.

"What kind of material bonds to living bone besides calcium phosphate?"

T. Kokubo

Various kinds of calcium phosphates have been found to bond to living bone and some of them are already clinically used as important bone substitutes. However, they are poor in mechanical strength, and hence cannot be used under load-bearing conditions. What kind of material can bond to living bone besides the calcium phosphate? According to our study, glass-ceramics A-W bonds to bone through an apatite layer which was formed on its surface in the living body. This apatite layer is formed on its surface even in an acellular simulated body fluid (SBF). This indicates that material able to form apatite layer on its surface in SBF can bond to living bone through the apatite layer which will be formed on its surface in the living body. It was found that even pure gel of SiO_2 , TiO_2 , ZrO_2 , Nb_2O_5 and Ta_2O_5 form the apatite on their surfaces in SBF. This indicates that materials base on these elements can bond to living bone, if their surfaces are a little modified. Apatite-forming abilities in SBF and bone-bonding ability *in vivo* were examined for various kinds of materials. As a result, it was found that Ti metal and its alloys with high fracture toughness's become to form apatite on their surfaces in SBF, and to tightly bonds to living bone, if their surfaces are a little modified with acid or alkali solution treatment and subsequent heat treatment. Some of them were found to induce bone formation even in muscle. These newly developed bioactive metals are believed to be important as bone-repairing materials which can be used even under load-bearing conditions.

"Bioactive Glasses: From Concept to Clinic, Lessons Learned"

L. Hench

In 1969 a new compositional range of soda-calcia-phospho-silicate glasses was discovered that formed a strong chemical bond with living bone. The composition that bonded fastest consisted of 45% (by weight) of SiO_2 -24.5% CaO -24.5% Na_2O -6% P_2O_5 . This unique material, 45S5 Bioglass, was the first man-made material to form a bond with living tissues. Studies of the reaction mechanisms of the bioactive glass surfaces *in vitro* and *in vivo* revealed eleven stages of reactions. The first stages lead to rapid HCA formation, leading to attachment, proliferation and differentiation of progenitor stem cells and bonding to both hard and soft connective tissues. Such bioactive glasses exhibit Class A bioactivity, with both osteoconduction and osteostimulation. FDA approved the first clinical products in the mid-80s with current applications in orthopedics, maxilla facial surgery, spinal surgery, and dentistry throughout the world. Molecular biology studies of the interaction of osteoprogenitor cells with bioactive glasses and the ionic dissolution products released from the glasses have identified seven families of genes that are activated or up-regulated by the stimuli that lead to rapid bone regeneration, a process the FDA approved to call osteostimulation. Studies of angiogenesis show that release of critical concentrations of ionic dissolution products from bioactive glasses can also lead to regeneration of vascular tissues. A significant healthcare application is use of bioactive glass as small particles in tooth paste. The bioactive glass phase leads to occlusion of exposed dentinal tubules and rapid re-mineralization of the teeth, prevention of tooth sensitivity and gingivitis. This revolutionary tooth paste is now available over the counter of millions of tubes have been sold in the last few years since its successful formulation. Issues encountered in transferring the concept of bioactivity to commercial success will be discussed.

Development of Multifunctional Nano-Bio Materials for Bioimaging and Si-rna Delivery

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Abstract

Bioactive glasses, doped with traces of copper (Cu) and zinc (Zn) were synthesized by fusion method. Cu and Zn present interesting functions for the biological metabolism through their antibacterial, anti-inflammatory and antifungal properties. Several physical methods were employed to characterize the bioactive glasses before and after immersion in a Simulated Body Fluid (SBF). The *in vitro* experiments showed that after soaking in SBF, the behavior of bioactive doped glasses are different compared to pure glass 46S6. Obtained results show that glass matrix undergoes some changes after 15 days of immersion. The non toxic character of doped glasses was confirmed after 24 hours of incubation. The kinetic of release of Cu and Zn was carried out. It highlights that Cu is more released than Zn. By SEM, the morphology of hydroxyapatite obtained with Zn-doped glass show a better crystallization compared to Cu-doped glass.

Keywords: Nano-biomaterials; LHRH peptide; siRNA delivery; Cell imaging; Cancer treatment; Multifunctionality

Acknowledgment

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Research Article

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Tracking of Bone Marrow Cells in Subcutaneous and Bony Implants with CM-DIL in a Mouse Model

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Abstract

Purpose: One major concern in bone tissue engineering is the fate of implanted cells. Data from the literature is not conclusive on the origin of the newly formed bone: newly formed tissues could derive from the grafted cells or from the host cells. A common cell-tracking technique consists in labelling cell membranes with fluorescent carbocyanine (CM-DIL), a molecule that remains stable for several weeks. It has been previously demonstrated that CM-DIL labelled cells could be detected 4 weeks after bony and sub-cutaneous implantation. The aim of the present study was to know whether grafted CM-DIL labelled cells from rat bone marrow could be detected subcutaneously in the calvaria of host nude mice at a time when osteogenesis occurs according to the literature that is 6 weeks and 8 weeks after implantation.

Materials and Methods: Unprocessed fresh Total Bone Marrow (TBM) was harvested from two Wistar rats and labelled with CM-DIL for 15 min. Then TBM was mixed with biphasic calcium phosphate ceramic particles and immediately implanted in 5 nude mice. For subcutaneous implantation (n=3), four pockets were created in the back of each animal. For the calvaria implantation (n=2), two defects (4 mm in diameter) were performed in the parietal bone and filled with smaller particles from the same manufacturer (size between 50 and 400 µm). In both groups, left side implants contained labeled cells whereas right side implants did not contain labeled cells. Animals were sacrificed after 6 and 8 weeks. Bone formation was qualitatively and quantitatively assessed by scanning electron microscopy (SEM), and histology. Tracking of donor cells was performed by confocal microscopy analysis.

Results: New bone formation was observed in all implants. We did not observe any difference between experimental and control groups. Labelled cells were detected both at 6 and 8 weeks in subcutaneous and calvarial implants. The CM-DIL signal was mostly detected in osteoblasts. Control implants did not show any signal at any time.

Conclusion: This study demonstrated that CM-DIL cell labelling does not alter the osteogenic potential of the grafted cells. Labelled cells can be easily detected both in bony and subcutaneous implants at a time when new bone formation is usually observed. We thus recommend CM-DIL cell labelling for the study of cell fate in bone tissue engineering.

Keywords: Cell-tracking; Nude mice; Total bone marrow; Bone regeneration

Acknowledgment

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Research Article

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Controlled Protein Delivery using Eggshell Derived Apatitic Nano Carriers with Alginate Coating

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Abstract

Therapeutic proteins have become the drugs of choice for treating numerous diseases due to advancements in biotechnology which has accelerated the economical and large-scale production of proteins. However, their unfavorable physicochemical properties necessitate novel biomaterials that can be more effectively protect and deliver protein drugs. Calcium Deficient Hydroxyapatite (CDHA) is an excellent candidate for protein delivery due to properties like biodegradability, high affinity to proteins etc. Eggshells are essentially made up of calcium carbonate with the presence of minor elements like sodium, strontium, magnesium etc., and can serve as abundant, cost effective natural resource for synthesis of CDHA. As the ions are under an optimal concentration for the mineralization, it is anticipated that the eggshell derived CDHA will have the appropriate properties for protein delivery applications. CDHA nanoparticles of different Ca/P ratios namely 1.51, 1.61 and 1.67 (hydroxyapatite) were synthesized using eggshell as well as with synthetic reagents and were well characterized. Loading and release profile of a model protein, bovine serum albumin (BSA) in the nanocarriers showed that the CDHA nanoparticles of Ca/P=1.51 has high loading and release of BSA compared to other samples. Maximum loading was observed for eggshell derived CDHA of Ca/P ratio 1.51 (57%) while synthetic CDHA of Ca/P ratio 1.51 showed a loading of 37%. Eggshell derived CDHA also showed greater release (25%) than synthetically derived CDHA (6.5%). To improve the release profile, alginate coated CDHA nanoparticles were synthesized and the experiments were repeated under same conditions. A maximum release percentage of 65% was observed for over a period of 2 days for alginate coated eggshell derived CDHA of Ca/P ratio 1.51. It may be concluded that eggshell derived CDHA nanoparticles of Ca/P ratio 1.51 can be ideal protein delivery agents and their release efficiency can be enhanced by coating with sodium alginate.

Keywords: Calcium deficient hydroxyapatite; Eggshell waste; Nanocarriers; Protein delivery; Bovine serum albumin; Alginate coating

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Research Article

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Producing a True Hydroxyapatite with the Necessary OH Content

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Abstract

Statement of Purpose: Hydroxyapatite (HAp) is widely used in orthopedics and dentistry for bone repair. The hydroxyl concentration impacts the properties of thermally processed hydroxyapatite. Therefore, the correct choice of processing conditions is essential to produce a composition with the desired properties.

Materials and Methods: Hydroxyapatite was synthesized using CaO and H_3PO_4 . Three different methods for the enhancement of [OH] in hydroxyapatite were used: 1) temperature of synthesis, 2) thermal treatment in different media (air vs. water vapor) at 1000-1200°C for 1-15 h., and 3) hydrothermal treatment. A previously developed [OH] quantification method using the [OH]/[PO₄] peak ratio obtained by Fourier transform infrared spectroscopy (FTIR) was used to study the influence of different processing parameters on the amount of [OH] groups in the structure of HAp. Phases and bonding was examined by X-ray diffractometry and FTIR.

Results: A higher synthesis temperature decreased the inclusion of CO₃ in the HAp structure and concurrently increased the structural order of apatite thereby directly influencing the amount of [OH] groups. Heating in water vapor at 1000°C for 15 hours produced significantly higher [OH] concentrations compared to thermal treatment in air. Hydrothermal processing of HAp at 200°C for 68 hours increases the amount of [OH] by about 10%.

Conclusions: A higher synthesis temperature provides more [OH] groups in the hydroxyapatite structure. Retention of structural water in the lattice requires steam during heating. The highest [OH] content in the hydroxyapatite structure is achieved with hydrothermal processing following thermal processing of the synthesized hydroxyapatite.

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Research Article

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Ultrathin Amorphous Calcium Phosphate Freestanding Sheet for Dentin Tubule Sealing

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Abstract

Novel dentin tubule sealing technique using ultrathin Amorphous Calcium Phosphate (ACP) freestanding sheet was developed. The ACP film was prepared by pulsed laser deposition technique and was separated from quartz substrate by dissolving photo resist as sacrifice layer. Sheets were pasted on polished human extracted teeth dentin by using calcium phosphate aqueous solution. The sheets kept wetted by artificial saliva for a few days. After drying Specimens, the bonding strength between the sheets and dentin were evaluated by quasi-tensile tests. A stainless rod of 3 mm in diameter glued on the ACP film was pulled by tensile tester via universal joint. The tensile strengths between the film and dentin were over 3 MPa, which is enough to seal dentin tubules. After tensile test, the specimens were embedded in epoxy resin and polished for investigation of bonding boundary nature by scanning electron microscope. The presence of a few microns thick bonding layer between the film and dentin was shown clearly by cross-sectional electron microgram. These results suggest that the dentin tubules maybe sealed by calcium phosphate sheet without organic materials.

Keywords: Pulsed laser deposition; Amorphous calcium phosphate; Freestanding film; Dentin hyper; Sensitivity

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Research Article

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Osteoblast Differentiation on HA Modified Zirconia an *In Vitro* Study

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Abstract

Objective: To evaluate and compare the effect of hydroxyapatite sputtered Y-TZP Zirconia on osteoblast cell attachment, proliferation, and differentiation.

Materials and Methods: Bone marrow cells from 5-week old male Wistar rat femur were cultured, passaged and then seeded on to pre-conditioned zirconia discs (density 2×10^5 cells/cm²; n=40; size=20 mm diameter) whose surfaces were modified first by sandblasting using Zirconia particles and then subjected to further modification by sputter coating hydroxyapatite followed by hydrothermal treatment. The surface roughness, morphology and chemical composition of the discs were evaluated. The Osteoblast cell morphology, the actin cytoskeleton and proliferation were analyzed after 3 hours, 6 hours, 24 hours and 72 hours of incubation. The Osteoblast differentiation was examined by staining the mineralized nodules after 14 days of culture. Transmission electron microscope was utilized to further elucidate the quality of nodules.

Results: The surface of the discs showed moderately irregular morphology ($R_a=0.49 \mu\text{m}$), after sandblasting. The thickness of hydroxyapatite coating was $1.7 \mu\text{m}$ and the morphology reflected the underlying blasted surface. The energy dispersive spectra of the samples pointed out the surface chemistry of the sandblasted surface was mostly based on the zirconium and oxygen, revealing high peaks on both the surfaces. The presence of calcium and phosphate was evident on the sputtered surface. The cell morphology showed globular cells initially with small cytoplasm extensions. At later time points cells were stellate-shaped with cell communications being established on the entire surface. The actin filaments showed spherical form in the early incubations time periods which extended to long and straight actin stress fibers at later time points. In this study both the surfaces show the expression of actin fibers indicating that surface roughness and composition have similar effect on the bone marrow cells. The sputtered hydroxyapatite surface revealed reduced cell growth at all-time points. Mineralization of the extracellular matrix, as the final stage of Osteoblast differentiation, showed no significant difference in nodule deposition on both surfaces.

Conclusion: Surface characteristics and composition contribute to the regulation of Osteoblast proliferation and mineralization eventually influencing osseointegration.

Keywords: Hydroxyapatite; Zirconia; Surface roughness; Cell morphology; Proliferation; Cytoskeleton

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Research Article

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Effect of Stressed and Unstressed Cell Culture Environment on the Viability of MC3T3 Cells with Calcium Phosphate

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Abstract

Fetal Bovine serum (FBS) is one of the main components of cell culture media (CCM). FBS provides cells with proteins they need to live as well as other factors and nutrients to growth. The effect of type of serum on cells has been previously studied (*Eslaminejad* 2009). Removing FBS from CCM creates a stressed cell culturing environment. A FBS withdrawal for 24 h is a challenge to cells as it may induce cells to undergo apoptosis (*Bai* 2006). Absence of FBS also allows studying the effect of different materials, or drugs, directly on the cells without the interference of the products found on the FBS. 50,000 MC3T3 cells were seeded on three different calcium phosphate (CaP) disc-shaped materials, i.e. GB 14, GB 9 and β -TCP, using a normally supplemented CCM in a 24-well plate. Normally supplemented CCM consisted of DMEM (PAA, Germany) with 10% FBS (PAA, Germany), 50 μ g/ml of ascorbic acid (SigmaAldrich, Germany), 5 mM β Glycerophosphate (SigmaAldrich, Germany), 2 mM L-Glutamine (Gibco, Germany) and 50 μ g/ml of Penicillin-Streptomycin (Gibco, Germany). GB14 and GB9 are glass-ceramics CaP materials. After 4 h, 24 h and 72 h –for some samples– supplemented CCM was changed by a media without FBS and this was let in contact with the cells for 24 h prior to analyzing the cell viability. Wells without materials, i.e. only cells, were used as controls. After the 24 h interaction passed, cell viability was measured. For all analyzed times cell viability was more important in CCM without FBS compared to those where FBS was present in CCM. It is interesting to notice that a stressed cell culture environment improved and maintained the cell viability of cells in contact with CaP materials. However, further *in vitro* and *in vivo* analyses to characterize the cellular fate.

Keywords: Cell culture media; Stressed; Unstressed; Cell viability; Glass-ceramics; Calcium phosphates

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Research Article

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Evaluation of Osteoinductive Properties of Different Combinations of Macroporous Biphasic Ceramic (MBCP+™), Simvastatin, Total Bone Marrow Cells and Rbmbp-2 in a Rat Subcutaneous Induced Membranes Model

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Abstract

Induced membrane technique has been proposed by Masquelet et al. (2003) for wide segmental diaphysal bone reconstruction with autologous cancellous bone graft. To reduce donor site morbidity following bone harvest, some authors proposed to add synthetic bone substitutes in combination or not with bone morphogenetic protein 2 (BMP-2) to the graft. To avoid the expenses of the growth factor, the use of the cholesterol lowering statins was investigated in numerous studies showing an enhanced expression of BMP-2 mRNA and osteoblastic differentiation *in vitro* and *in vivo*. Therefore, statins were proposed as BMP-2 substitutes. The aim of this study was to evaluate the osteoinductive capacity of simvastatin in comparison to BMP-2 in an induced membrane filled with the bone substitute MBCP+™ (Biomatlante) in association with total bone marrow allografts in an ectopic site of rats.

Therefore, subcutaneous induced membranes were filled with different combinations of MBCP+™, total bone marrow, simvastatin, and rhBMP-2 (Inductos®). Half of the samples were analyzed by microCT and histology. The other half was crushed for protein extraction, Western Blot quantification of BMP-2 and VEGF, and cell proliferation and differentiation of hBMSCs cultures. Furthermore, *in vitro* studies have evaluated simvastatin toxicity and early osteoblastic differentiation on rBMSCs cultures.

Simvastatin caused no cytotoxicity at 10⁻⁶ M and demonstrated early osteoblastic differentiation of rBMSCs by ALP assays and qRT-PCR for Osteocalcin and BMP2. Supernatants of crushed induced membranes initiated early osteoblastic differentiation of hBMSCs.

MicroCT analysis did not reveal significant differences between every conditions. Histology demonstrated higher scaffold cellularity for conditions with bone marrow cells. BMP-2, but not simvastatin induced osteoid formation.

In conclusion, simvastatin showed to affect osteoblastic differentiation, but didn't improve osteoinductive properties of the biomaterial in induced membranes. According to our experience, BMP-2 stays the state of the art in osteoinductive studies *in vivo*.

Keywords: Induced membranes; Bone substitute; Simvastatin; BMP-2; Bone marrow cells; Tissue engineering

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Research Article

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Development and Characterization of Novel Bioactive Chitosan/Gamat Composite Films for Tissue Repair Purposes

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Abstract

Chitosan and gamat (sea cucumber) have proven wound healing properties individually and yet they have never been used together. We are interested to explore the prospect of this combination that we expect may show improvement in wound healing and tissue regeneration activities. Thus, the present research work concentrates on the development of chitosan/gamat composite films that can release gamat extract over an extended period of time and also in a controlled manner. A solution of different percentages of chitosan, is prepared in lactic acid, followed by addition of glycerol as plasticizer and differing amounts of gamat powder. Prepared composite films will be characterized for their physical and mechanical properties. They will be evaluated for thickness, folding endurance, in vitro drug release by diffusion studies, water absorption capacity, tensile strength and in vivo wound healing studies by using albino rats. It is expected that chitosan/gamat composite films will show more/improved wound healing property than chitosan blank film without interfering in strength of film.

Keywords: Chitosan; Gamat; Wound healing; Tissue regeneration; Composite films; Thin films

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Research Article

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Effect of Additive on Properties of Injectable Hydroxyapatite/Collagen Paste Using Sodium Alginate

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Abstract

Artificial bones composed of bioactive ceramics are widely used in medical and dental fields in the forms of dense and porous bodies, granules and pastes. Pastes have advantages of fluidity to be used in minimally invasive surgery and formability to fit to the bone defect with complicated shape. Present bone pastes are composed of hydroxyapatite; thus, paste remains long time to be a potential risk of fracture. Therefore, biodegradable bone paste is strongly desired by surgeons. On the other hand, hydroxyapatite/collagen bone-like nanocomposite (HAp/Col) synthesized by Kikuchi et al. is incorporated into bone remodeling process similar to autologous bone graft. This biological property is suit for biodegradable bone paste. Kochi et al. had fabricated bone paste using the HAp/Col and sodium alginate (Na-Alg), and defined the optimum conditions to prepare paste; however, the paste did not exhibit sufficient anti-decay property in phosphate buffered saline.

In the present paper, an additive, organic acid or calcium compound, was mixed in the Injectable HAp/Col paste to control Ca^{2+} ion concentration in the paste, and influence of additives on hardening properties of the paste were investigated. The HAp/Col at the HAp and collagen ratio of 4:1 was prepared by simultaneous titration method (Kikuchi et al., Biomaterials, 2001). The HAp/Col paste was fabricated at the optimum conditions, powder liquid ratio of 0.6 and the weight ratio of HAp/Col and Na-Alg of 9:1. One additive was chosen from organic acids or calcium compounds, and mixed with the HAp/Col paste. The paste prepared was served for viscosity and decay property test.

Additive influenced in increasing of both viscosity and decaying time of the paste, but did not realize sufficient anti-decay property. The results suggested that further improvement of releasing rate of Ca^{2+} ion would realize the paste with optimal viscosity, hardening and anti-decay properties.

Keywords: Hydroxyapatite/collagen bone-like nanocomposite; Paste; Sodium alginate; Organic acids; Calcium compounds; Hardening properties

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Research Article

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Efficacy of HA-coated One-piece Implants on the Graft less Procedure of Immediate and Early Implant Placement for the Anterior Maxilla

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Abstract

Introduction: Inadequate alveolar bone volume is a common limitation in the anterior maxilla since bone resorption takes place soon after tooth extraction. Such problems have been overcome by bone grafting or GBR. But they are invasive procedures and need long treatment period.

Purpose: The aim of this study was to evaluate the efficacy of HA-coated one piece implants on the graft less procedure of immediate and early implant placement for the anterior maxilla.

Materials and Methods: Thirty patients, 22 males and 8 females, 27 to 67 years of age, were included in this study. Thirty-four hydroxyapatite-coated one-piece implants (AQB implant®, ADVANCE, Co. Japan) were placed immediately or early within 8 weeks after falling teeth had been removed. All implants were placed and positioned slightly to the palatal aspect, with the exposed implant surface clearly inside the alveolar crest. Some of them left craterlike defect on the facial aspect of the implant. In cases bone chips were collected from the implant bed during drilling, they were placed on the facial aspect of the implant. No donor site was prepared. No membrane and filling materials were used. Following items were investigated.

- 1) Sex and age distribution,
- 2) Location of implants inserted,
- 3) Diameter and length of implants used,
- 4) Duration from tooth extraction to implant placement,
- 5) Duration of healing period from implant placement up to addition of occlusal force.

The survey was carried from January 2005 through February 2013.

Results: All 34 implants were functionally loaded and showed no sign of morbidity and per implant disease.

Conclusion: HA-coated one piece implants were found to be effective on the graft less procedure of immediate and early implant placement for the anterior maxilla.

Keywords: Graft less procedure; anterior maxilla; HA-coated implant; One-piece implant; Immediate and early placement; Bone graft

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Research Article

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Biomimetic Apatite's: Surface State and Interfacial Considerations in Link with Bone Regeneration

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Abstract

Biomimetic nanocrystalline appetites occupy an increasingly appealing position in view of bone tissue engineering applications due to their high analogy to bone mineral, in terms of chemical composition, nonstoichiometry, (micro) structure, surface state and reactivity. The specificities of apatite nanocrystals, either composing bone mineral or their synthetic analogs, will first be recalled in this contribution. These include very peculiar physico-chemical features, especially on the surface of the nanocrystals where a non-Apatitic calcium phosphate layer is present and controls most of the surface and interfacial properties. In a second part, the main types of interfaces of interest involving apatite nanocrystals intended for biomedical applications will be overviewed and commented. The interaction with surrounding aqueous media will be particularly examined, underlining the nanocrystals ageing in solution (accompanied by significant variations in the physico-chemical characteristics of the nanocrystals, in terms of crystallinity state, hydration level, mean crystallite dimensions, extent of non-Apatitic ionic environments...) as well as surface reactivity features. The subjection of such nanocrystals to vacuum or air during drying processes will also be examined, pointing out some surface amorphization, the consequences of which (e.g. in terms of increased surface ion release after re-immersion) will be commented. The impact of synthesis/post-synthesis parameters on the nature and characteristics of apatite compounds obtained will be illustrated in relation with potential industrialization aspects.

Keywords: Biomimetic appetites; Surface and interface; Surface reactivity; Bioactive ceramics

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Research Article

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Comparison of Two Composites Developed To Be Used As Bone Replacement-PMMA/Bioglass 45S5® Microfiber and PMMA/Hydroxyapatite

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Abstract

A development of polymethylmetacrylate (PMMA)-45S5 bioglass® microfiber composite with porosity for application as bone scaffolds was performed. PMMA is the most used biomaterial of the bio-inert type however; it presents a low elastic modulus and mechanical strength compared with the bone limiting its use. The 45S5 bioglass® bioactivation index is superior to 8 which indicates ability to integrate with bone tissue and even soft tissues nevertheless, is too fragile. This proposal is innovative and has been based on previous work of this research group based in porous PMMA with hydroxyapatite (HA) that showed excellent results of biocompatibility but low mechanical strength. The current concept introduces 45S5 bioglass® microfibers instead HA hoping to obtain a bone scaffold with a higher mechanical strength keeping good bone integration. In order to evaluate the performance of this biomaterial compression tests were carried out as well as *in vivo* tests for the assessment of bone-material integration. The scaffolds were implanted at tibia of male adult rats. The animals were divided into four study groups: defect control, material control, PMMA/HA and PMMA/45S5 Bioglass®. After 60 days of implantation all PMMA/45S5 Bioglass® scaffolds show a good compatibility without any sign of inflammation. In the PMMA/HA study group after 60 days of implantation, three tibiae show a significant bone deformity. According to compression tests conducted PMMA dense samples have a much higher mechanical strength (near to 50 MPa) than the samples of porous PMMA, PMMA/HA and PMMA/45S5 Bioglass® as was expected. However the addition of HA or Bioglass® significantly increase the mechanical strength of the sample of porous PMMA. The PMMA/45S5 bioglass® show an excellent biocompatibility without extreme compromising its mechanical strength.

Keywords: Bone scaffold; Polymethylmethacrylate; Biocomposites; *In vivo* test; Compression test; Scanning electron microscope image

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Research Article

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Evaluation of the Capacity of Bio-inspired Carbonate Apatite Composite Bone Substitute to Support Bone Regeneration in Goat Model

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Abstract

Bio-inspired design of correlative bone substitute is very important to induce new bone formation within physiological condition. Inter-connective porous composite with Ca/P ratio closer to the original bone with the ability to promote bone formation and facilitate mass transfer management in the body is a critical aspect in the design of bone substitute. In this study, we have developed carbonate apatite composite with nano-dispersed Calcium-Phosphate in gelatin. In the current study, carbonate apatite composite was fabricated by synthesis of apatite in gelatin system and physically cross linked to result composite with 150 μm pore networks with controlled biodegradable manner. A physical, mechanical, and chemical characterization of the composite was confirmed for 3 different composite compositions, those are CHA-50, CHA-25, and CHA-00. Implantation of the 3 different fabricated composites was done in the humerus bone of local Ettawa goats for 2, 4, 8, and 12 weeks with carbonate apatite blocks were used as the control of the osteoconductivity evaluation. It was found that no significant differences on the degradability of the composites with different apatite contents, meanwhile CHA-50 may probably the most promising composition in terms of its osteoconductivity. It was also proved that 150 μm pore networks are considered reliable scaffold construction for bone tissue engineering. Early clinical trial is now in progress in our dental hospital.

Keywords: Bio-inspired carbonate apatite; Composite; Bone substitute; Properties; Osteoconductivity; Goat model

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Research Article

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Self-Organized Magnesium Carbonate Apatite/Collagen 3D-Scaffolds for Bone Regeneration

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Abstract

Synthetic bone substitutes with nanostructure and physico-chemical properties similar to natural bone has been produced to potentially reconstruct damaged or lost bone tissue. The aim of this study was the development and characterization of a synthetic 3D scaffolds of magnesium carbonate apatite /anionic collagen composite (MCA/col) for tissue engineering applications. The scaffolds were prepared by simultaneous addition of calcium, magnesium and collagen solutions to carbonate and phosphate buffer solutions at 37°C, pH=8. They were cross-linked with 0.25% (v/v) glutaraldehyde (GA) solution and freeze-dried. Physico-chemical (XRD, FTIR, TGA, ICP, BET), *in vitro* tests (dissolution tests, cytotoxicity and Osteoblast cell culture) and morphological (micro-CT, OM, SEM, TEM) characterizations were performed. FTIR spectrum confirmed the presence of collagen, phosphate and type B carbonate absorption bands. The mineral phase as well as Mg contents were estimated by TGA and ICP, respectively, and are close to the bone contents. The composite sections stained by Von Kossa showed a mineral deposit on collagen fibers. After the mineralization process, open pores structures with interconnectivity was observed by SEM. ICP estimated the amount of Ca, Mg, and P ions released in acid buffer solution after 180 minutes. Mg ions in the acidic buffer reached saturation within 120 minutes while the concentration of the Ca and P ions continued to increase after this time. The chemical cross-linking with GA was effective to stabilize the scaffolds under wet conditions and showed no *cytotoxicity* effects based on three assays parameters. The scaffolds maintained their structures after 14 days in culture medium presenting a continuous layer of human Osteoblast cells at the time of incubation. *Bone cells* (SaOs2) adhered, and proliferated on the 3-D scaffold MCA/col. In resume, it seems that these 3-D composites mimicking the bone structure could be useful as temporary substitutes for bone replacement.

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Research Article

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Fabrication of Sr-Substituted Hydroxyapatite Thin Film by Sputtering Technique

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Abstract

This study describes the preparation of Sr-substituted hydroxyapatite thin film by sputtering technique and osteocompatibility of the films by cell culture. The mixture of Hydroxyapatite (HA) and Strontium Apatite (SrAp) were used as a sputtering target. The HA and SrAp powders were mixed at 0, 20, 40, 60, 80, and 100 at% of Sr/(Sr+Ca) ratio. The films were coated from mixture targets of HA and SrAp by the sputtering. Then the films were by X-ray Diffract meter (XRD) and Scanning Electron Microscopy (SEM). The Sr/ (Sr+Ca) molar ratio of the film was observed by measuring the dissolved film solution using inductively-crystallized by a hydrothermal treatment to reduce the dissolution of the films. The films were characterized coupled plasma atomic emission spectrometry (ICP). The adhesion strength of the film to the substrate was measured by a pull-out test. The osteocompatibility of the films was also evaluated by bone formation area of the Osteoblast cells.

From the XRD measurements, the diffraction peaks shifted to lower 2θ values with increasing Sr/(Sr+Ca) ratios of the target, which showed Sr incorporation into HA lattice. From the ICP measurements, the Sr/ (Sr+Ca) ratio in each film was almost the same ratio to that of the target. It suggests that the Sr/(Sr+Ca) ratio of the films can be controlled by changing the Sr/(Sr+Ca) ratios of the target. From the pull-out tests, the adhesion strength of the films to the substrate tended to increase after the hydrothermal treatment. In the cell culture, the films with 20-80% of Sr/(Sr+Ca) showed higher bone formation area than the film with 0% of Sr/(Sr+Ca) corresponding a conventional HA film. These results showed that Sr incorporation into HA may lead to increase the osteocompatibility of the HA film.

Keywords: Sputtering; Hydroxyapatite; Strontium; Osteoblast cell

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Research Article

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Improvement of Mechanical Properties of 3D Printed Hydroxyapatite Scaffolds by Polymeric Infiltration

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Abstract

Tailor made ceramic scaffolds in combination with the corresponding surface chemistries and biology is of great importance for successful implantations and guarantees a rapid osseo-integration. This project investigates the infiltration of 3D-printed hydroxyapatite scaffolds with biodegradable polymers. Hydroxyapatite is one of the major constituents of the inorganic component in human tissues such as bones and teeth. A suitable infiltration technique was developed to improve mechanical properties of the printed ceramic specimens. To perform the infiltration biopolymers for instance gelatine, Polycaprolactone (PCL), Polyvinyl Alcohol (PVA) and others were utilized. The mechanical characterization was performed by compressive strength measurement. Further analysis was performed by porosity measurement according to Archimedes, Mercury Intrusion Porosity (MIP) and Scanning Electron Microscope (SEM). Post treated samples showed a significantly increased compressive strength compared to untreated samples. The most promising results were observed after multiple gelatine infiltration. This combination of biodegradable polymer and bioactive ceramic processed with an optimized 3D-print and infiltration method results in customizable scaffolds with promising mechanical properties. As an outlook, the developed infiltration methods allow the integration of bioactive compounds like bone collagen or anti-infectious agents such as antibiotics or silver/copper ions.

Keywords: Infiltration; 3D-printing; Biodegradable polymer; Hydroxyapatite; Scaffold; Regenerative medicine

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Research Article

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Fabrication of α -Tricalcium Phosphate Cement using Chelating Mechanism of Inositol Phosphate and its Histological Evaluation

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Abstract

Calcium-Phosphate Cement (CPC) has received much attention due to its handling property during operation. We have previously developed a novel CPC consisting of α -tricalcium phosphate (IP6- α -TCP cement) using chelating capability of Inositol Phosphate (IP6). In the present study, adsorption and desorption behaviors of IP6 to/from α -TCP powders were investigated. Afterward, the IP6- α -TCP cements were prepared using sodium citrate (Cit) and sodium alginate (Alg) as liquid components of the cement; and, effect of additions of Cit and Alg on the handling and anti-washout properties of IP6- α -TCP cement was examined. The single-phase α -TCP powder was prepared by ball-milling using ZrO₂ beads for 120 min (α -TCP120). Amount of IP6 adsorbed on the surface of α -TCP120 in 1000 ppm IP6 increased time-dependently and reached saturation at more than 6 h. Desorption of IP6 from IP6- α -TCP120 powder was constant level up to day 7 in both acetate (pH 5.5) and HEPES (pH 7.0) buffers, and no changes in crystalline phase and morphology were observed after the desorption tests. Compressive strength (CS) of the IP6- α -TCP120 cements fabricated with 0.5 and 0.7 mol·dm⁻³ Cit containing 1 mass % Alg was higher than those of 0.5 and 0.7 mol·dm⁻³ Cit without Alg. The IP6- α -TCP120 cement paste using Cit containing Alg improved the handling property of the pastes, and showed resistance against washout in pure water, implying that Cit and Alg interacted with IP6- α -TCP120 powder and the CSs of cements were consequently enhanced. *In vivo* study implanted IP6- α -TCP120 cement into a pig tibia for 4 weeks indicated the direct contact of the cement with newly-formed bones without fibrous tissue layers. The IP6- α -TCP120 cement is promising for application as a novel paste-like artificial bone.

Keywords: α -Tricalcium phosphate; Calcium-phosphate cement; Bone graft; Inositol phosphate; Biodegradability; Biocompatibility

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Research Article

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Hemocompatibility of Three Kinds of Putties Used in Bone Regeneration and Tissue Engineering

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Abstract

Actually numerous bone substitutes are based on the combination of Hydrogel and Bioceramic. These resorbable bone substitute putties support and regenerate bone tissue after implantation. The efficiency of these multiphase materials is due to the osteogenic and osteoconductive properties of the micro porous biphasic calcium phosphate Bioceramic. The associated hydro soluble polymers are considered as carriers in order to achieve the rheological properties of putty mouldable bone substitutes.

In order to test the efficacy and performance of such biomaterials, in vivo tests are performed; they are based on histomorphometry, bone regeneration and Bioceramic resorption. But very often the "biomaterials researchers" do not take into account safety requirements for CE mark or FDA early enough. Particularly in the case of putty biomaterials, it is relevant to demonstrate the absence of unwanted interactions with blood. The purpose of this paper is to present the hemolytic property of 3 materials combining hydrogel and Bioceramic granules according to ASTM standard F756-00 and NF ENISO 10993 tests.

The same granules were used for the 3 putties. It was micro macroporous biphasic calcium phosphate (HA60% and β -TCP 40%). The differences are the hydrogel. In sample A, it is a non reticulated hydro soluble cellulosic ether HPMC (In'Oss™), in sample B, it is a thermo reversible poloxomer type Pluronic F127 and in sample C, it is reticulated hydrogel obtained by irradiation. Fresh human blood was used, using a colorimetric method. The test was realized in triplicate. The hemolytic index IH was determined using the formula: $IH (\%) = \frac{\text{Free hemoglobin (g/l)} \times 8 \times 100}{\text{Total hemoglobin}}$

According to the test, a medical device is non hemolytic if IH is between 0-2%, low hemolytic if IH is between 2-5%, and hemolytic if IH is higher than 5%.

	Sample A	Sample B	Sample C
Hemolytic Index	1.2 ± 0.6	1.2 ± 0.9	1.4 ± 0.5
Hemolytic grade	Non hemolytic	Non hemolytic	Non hemolytic

According to the test, the 3 kinds of putty were non hemolytic. This test is simple, easy to realize and represents a good pre-evaluation of scaffolds used in bone regeneration and/or tissue engineering.

Keywords: Putty; Bioceramic; Hemocompatibility; Bone substitute; Composite; Hydrogel

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Research Article

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PMMA/Ag Bone Cements: Evaluation of Bioactivity, Biocompatibility and Antibacterial Properties

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Abstract

A new biomaterials generation was born by combining bioactivity, biocompatibility and antibacterial properties of materials, either by antibiotic or silver loading. Silver based antimicrobials capture much attention not only because of the non toxicity of the active Ag⁺ to human cells but because of their novelty being a long lasting biocide with high temperature stability and low volatility. The antimicrobial efficacy of these composites depends on their ability to release the silver ions from these composites upon interaction with biological fluids [1,2]. Hence, silver doped materials are use as an alternative (or complementary) to antibiotic loaded cements, We have developed in this work a new strategy for orthopedic/dental implants based on both concepts improvement: bioactivity and antibacterial activity by incorporating different concentration of Ag₂O in PMMA bone cement followed by collagen electrodeposition. The study is focused on scanning electron microscopic analysis before and after immersion in SBF and complementary ATRFTIR spectroscopy which evidenced the phosphate characteristic bands of hydroxyapatite, depending on the silver oxide content in the samples. Biocompatibility tests were performed in human fibroblasts culture and interpreted in terms of adherence and proliferation extent with respect to the presence of collagen coating and silver concentration in samples.

Keywords: Acrylic bone cement; Silver; Bioactivity; Biocompatibility; Antibacterial

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Research Article

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Apatite Formation on Titanium Containing Apatite

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Abstract

Hydroxyapatite (HAp) is one of the components of human bone and the HAp ceramics are used by bone substitutes. But mechanical properties of the HAp ceramics were not fitted at human bone due to the brittleness. Thus some composite materials consisting of HAp and other compounds, and tricalciumphosphate ceramics as biodegradable materials have been investigated. Some titanium containing apatite (TMA) ceramics prepared by wet synthesis was superior to HAp ceramics of mechanical property and cutting performance. In this study, we investigated chemical reaction of TMA in body environment. Each commercial titanium containing apatite powder (TMA) or commercial Hydroxyapatite Powder (HAp) was formed to circular pellets by uniaxial pressing. The specimens were heated in muffle furnace to 1000°C at a rate of 20°C/min, and kept for 10 h, followed by cooling to room temperature at the natural cooling rate of the furnace. These TMA and HAp specimens are denoted as TMA1000 and HAP1000, respectively. Some TMA1000 specimens were soaked in NaOH solution at 60°C for 6 hours, dried at 40°C for 24 hours, and heated at 400°C for 1 hour. These alkali treatment specimens are denoted as TMA-AT. TMA1000, HAP1000, and TMA-AT were soaked in simulated body fluid (SBF: Kokubo's solution) at pH 7.25 at 37.0°C up to 14 days. The surfaces of the samples before and after soaking in SBF were characterized by X-ray Diffraction (XRD), and Scanning Electron Microscopic (SEM) observation, and Inductively Coupled Plasma (ICP). After soaking in SBF for 14 days, some particles were precipitated on the surface of TMA-AT and HAP, but were not precipitated of TMA1000. These particles were estimated by HAp by SEM and changes at Ca and P concentration in SBF. The TMA ceramics are expected to be a new bone or dental substitutes.

Keywords: Titanium containing apatite; Apatite formation; Simulated body fluid; Surface treatment

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Research Article

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Multiphasic Gene Activated Matrices for Osteochondral Regeneration in Osteoarthritis

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Abstract

The aim of this work is to develop a gene-activated multiphasic matrix targeting the regeneration of musculoskeletal tissues affected in osteoarthritis. Layering of the different composites and the activation of gene expression "on demand" via a heat-inducible plasmid by an alternating magnetic field are tools for the spatiotemporal control of gene expression essential for targeting the different tissues involved. Therefore, we assessed the suitability of a Thermoresponsive Hyaluronan Hydrogel (THH) as carrier for smart bioceramics and Magnetic Nanoparticles (MNPs) via rheological analysis, Differential Scanning Calorimetry, release of ¹²⁵I-labeled Adenoviruses (AdV) and gene transfer efficiency of AdV vectors using rat Mesenchymal Stem Cells (MSCs) and detecting the expression of the reporter gene dsRed by fluorescence microscopy of outgrowing cells 48 h after assembly of gels.

Rheological analysis demonstrated that composites including THH and MNPs in a therapeutically relevant amount still display temperature induced sol-gel transition. Even at the highest particle content viscoelastic shear moduli of the composites was <100 Pa, making them injectable through a cannula. Differential Scanning Calorimetry confirmed the trend of the rheology data, showing phase transition in a physiologically relevant temperature at 30 ± 2 °C. Release of ¹²⁵I-labeled AdV as determined by gamma counting was retarded for THH including MNPs compared to pure THH. Thus THH containing MNP might serve as a depot for AdV delivery. Infectivity of AdV vectors towards MSCs was retained within THH/MNP composite.

We have demonstrated that THH are suitable carriers for and selected MNPs, and that AdV maintain their infectivity within such a matrix. These features combined the possibility of layering different layers through injection make these composites suitable candidates for the regeneration of different tissues involved in osteoarthritis.

The research leading to these results has received funding from the European Union's 7th Framework Program under grant agreement n° NMP3-SL-2010-245993.

Keywords: Magnetic nanoparticles; Hydrogels; Gene delivery; Composites; Spatiotemporal control; Osteoarthritis

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Research Article

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Fabrication of Interconnected Porous Carbonate Apatite from Gypsum Spheres

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Abstract

Interconnected carbonate apatite may be one of the ideal bone replacements. In this study, gypsum spheres were used for the fabrication of interconnected porous carbonate apatite bone replacement. First, spherical gypsum with approximately 1 mm in diameter was fabricated by w/o emulsion method. In short, calcium sulfate hemihydrates ($\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$) slurry was dropped into oleic acid and stirred at room temperature. The set spherical was calcium sulfate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The spherical gypsum was heat-treated to convert its conversion from dihydrate to hemihydrates to obtain self-setting ability. The spherical calcium sulfate hemihydrates were immersed in water to allow setting reaction between calcium sulfate hemihydrates spheres based on dissolution-precipitation reaction. As a result of setting reaction, interconnected porous gypsum block with 60% porosity was fabricated. The compressive strength of the fully interconnected gypsum block thus prepared was approximately 700 kPa. Then interconnected porous carbonate apatite bone replacement was fabricated using fully interconnected gypsum block as a precursor based on dissolution-precipitation method using a mixed solution of Na_2HPO_4 and NaHCO_3 at 120°C . The composition was B-type carbonate apatite in which PO_4 is replaced to CO_3 . Porosity was approximately 80% and the compressive strength was 300 kPa. We concluded that gypsum may be good candidate to fabricate interconnected carbonate apatite block.

Keywords: Carbonate apatite; Interconnected pore; Gypsum

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Research Article

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In Vitro Testing of Titanium Substrates Coated with Biomimetic Calcium Phosphate

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Abstract

Titanium implants may be coated with calcium phosphates by a biomimetic precipitation method, in order to improve their biocompatibility. Previous studies have indicated the potential of a biomimetic method based on a simplified solution with calcium and phosphorus ions. The present work aimed to assess the biocompatibility by *in vitro* tests of titanium substrates coated with biomimetic calcium phosphate precipitated from the simplified solution. As substrates, commercially dense titanium sheet and macroporous titanium samples produced by powder metallurgy, both ASTM F67 grade 2, were used as controls. These substrates were submitted to chemical and heat treatment for enhancing surface bioactivity and then immersed in the simplified solution for 10 days. Samples characterization was performed by Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS), X-Ray Photoelectron Spectroscopy (XPS) and *in vitro* tests. Calcium phosphate coatings were deposited on both substrates, as indicated by SEM/EDS and XPS analyses. The *in vitro* evaluation showed that the amount of cells was more significant in cultures grown in titanium substrates with calcium phosphate coating, compared with control substrates, since the results indicated an increase in osteoblasts adhesion and proliferation. Further, biomimetic coating precipitation on titanium substrates affected the osteogenesis parameters, including alkaline phosphatase and nodules formation similar to bone by mineralization process.

Keywords: Titanium; Calcium phosphate; Biomimetic; *In vitro* testing; Powder metallurgy

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Research Article

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Bioinspired Apatite-Citrate Nanocrystals: Synthesis, Characterization and Applications

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Abstract

Nanocrystalline apatites constitute the main inorganic part of mammalian hard tissues, and an increasing interest in the preparation of synthetic equivalents, so-called "biomimetic", able to specifically mimic the morphological and physical-chemical features of biological apatite is emerging. One of the most interesting strategies to prepare well defined nano-apatites is their structural control using biocompatible calcium-chelating agents during the synthesis. Citrate ions are biocompatible calcium complexing agents, inhibitors of apatite crystals growth and they are present in the organic bone matter up to 5% wt. Thus, the synthesis of apatite with citrate ions can be a very powerful strategy inspired from nature to control the chemical-physical features of the final product. Novel apatite-citrate nanoparticles with mean lengths ranging from 20 to 200 nm were synthesized by thermal-decomplexing batch method (Delgado-Lopez et al. Acta Biomater, 2012). The role of the carbonate in the precipitation medium was studied and needle-like and plate-shaped morphologies were obtained in the absence and in the presence of carbonate in the reaction mixture, respectively. The precipitation time and the presence of carbonate strongly affected the apatite composition, as well as the dimensions and crystallinity of nanoparticles. The nanoparticles were composed of a well-ordered apatitic core embedded in a non-apatitic hydrated layer containing citrate ions. This layer progressively transformed into more stable apatite domain upon maturation in aqueous media. IR spectroscopy was used to investigate the surface hydration of materials, in terms of adsorbed water molecules and surface hydroxyl groups, as well as the Lewis acidity of surface cations, by removing water and adsorbing CO. The prepared apatite-citrate nanocrystals are promising materials to be used as carriers for drug delivery systems as well as building blocks for the preparation of nanostructured scaffolds in bone tissue engineering. In this respect, their use as anticancer (doxorubicin) delivery system was also studied.

Keywords: Biomimetic apatite; Nanocrystals; Citrate; Drug delivery; Doxorubicin

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Research Article

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Novel Method for the Synthesis of β -TCP Coatings with Tailored Physico-Chemical Properties on Zirconia Bioceramics

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Abstract

Zirconia implants are becoming increasingly important in the field of dental medicine because of their good mechanical properties, biocompatibility, and for aesthetic reasons. However, zirconia is bioinert, and this can lead to poor fixation of the ceramic implant in the bone. A common approach to improving the osseointegration of load-bearing implants is the deposition of a bioactive calcium phosphate layer on their surface. Among the known calcium phosphate phases, the β -tricalcium phosphate (β -TCP) phase is especially attractive due to its reported clinical success [1].

We have developed a novel method for the preparation of β -TCP coatings with tailored physico-chemical properties, i.e., surface topography, mechanical strength and dissolution rate. It involves two steps: i) the rapid, wet-chemical deposition of a biomimetic CaP coating and ii) the subsequent post-deposition processing of the deposited biomimetic CaP coating, which includes a heat treatment above 800°C, followed by a short sonication (≈ 1 min) in a water bath. The obtained β -TCP coatings were thin and displayed a roughness in the nanometer range. By changing the heating regime, i.e., the heating rate, temperature and dwell time, the properties of the β -TCP coatings could be controlled. The β -TCP coating that was prepared by heating at 800°C (β -TCP-800) was 500 nm thick and was composed of nanosized crystals that formed a reticular porous structure ($R_a = 46 \pm 5$ nm). The β -TCP-900 coating was composed of grains and had a dense morphology ($R_a = 42 \pm 5$ nm). It displayed the lowest dissolution rate in physiological solution and possessed the highest mechanical strength (tensile strength = 52 ± 4 MPa; critical load during scratch testing = $L_c = 97 \pm 9$ N). Heating above 900°C produced β -TCP coatings with increased thickness, surface roughness ($R_a = 200$ -400 nm) and dissolution rate in a physiological solution, but a reduced mechanical strength. The prepared β -TCP coatings exhibited an apatite-forming ability in simulated body fluid and increased the adsorption of serum proteins on the zirconia substrate.

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Keywords: Zirconia; β -tricalcium phosphate; Coating; Dissolution; Adhesion; Bioactivity

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Research Article

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Effect of Additive on Properties of Injectable Hydroxyapatite/Collagen Paste Using Sodium Alginate

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Abstract

Artificial bones composed of bioactive ceramics are widely used in medical and dental fields in the forms of dense and porous bodies, granules and pastes. Pastes have advantages of fluidity to be used in minimally invasive surgery and formability to fit to the bone defect with complicated shape. Present bone pastes are composed of hydroxyapatite; thus, paste remains long time to be a potential risk of fracture. Therefore, biodegradable bone paste is strongly desired by surgeons. On the other hand, hydroxyapatite/collagen bone-like nano composite (HAp/Col) synthesized by Kikuchi et al. is Incorporated into bone remodeling process similar to autologous bone graft. This biological property is suit for biodegradable bone paste. Kochi et al. had fabricated bone paste using the HAp/Col and sodium alginate (Na-Alg), and defined the optimum conditions to prepare paste; however, the paste did not exhibit sufficient anti-decay property in phosphate buffered saline.

In the present paper, an additive, organic acid or calcium compound, was mixed in the injectable HAp/Col paste to control Ca^{2+} ion concentration in the paste, and influence of additives on hardening properties of the paste were investigated. The HAp/Col at the HAp and collagen ratio of 4:1 was prepared by simultaneous titration method (Kikuchi et al., Biomaterials, 2001). The HAp/Col paste was fabricated at the optimum conditions, powder liquid ratio of 0.6 and the weight ratio of HAp/Col and Na-Alg of 9:1. One additive was chosen from organic acids or calcium compounds, and mixed with the HAp/Col paste. The paste prepared was served for viscosity and decay property test. Additive influenced in increasing of both viscosity and decaying time of the past, but did not realize sufficient anti-decay property. The results suggested that further improvement of releasing rate of Ca^{2+} ion would realize the paste with optimal viscosity, hardening and anti-decay properties.

Keywords: Hydroxyapatite/collagen bone-like nanocomposite; Paste; Sodium alginate; Organic acids; Calcium compounds; Hardening properties

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Research Article

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Bioavailability of Strontium Ions from Bioactive Glasses in Vivo: A Micro-Pixe Study of Trace Elements at the Bone Interface

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Abstract

Recent advances in the field of biomaterials are the development of bioceramics able to release osteoinductive ions directly onto the site of implantation. The osteoinductive ions locally delivered help increase ontogenesis through the direct stimulation of the Osteoblast activity. Of special interest is the delivery of strontium (Sr) ions, since Sr has marked stimulatory effects onto bone cells resulting in strengthening of bone, stimulation of bone formation and decrease in bone resorption [1]. The *in vitro* behavior of strontium-doped bioactive glasses is in particular promising, due to their high chemical reactivity associated to the release of physiological concentrations of osteoinductive ions [2]. Then studying the local release of Sr traces *in vivo* is of key interest and would be a step forward, but calls for a highly sensitive technique besides providing an excellent (micronic) spatial resolution. In this context nuclear microprobes such as the PIXE (Particle-Induced X-ray Emission) technique, clearly appear as powerful tools of investigation [3].

Here the *in vivo* behavior of a Sr-delivering bioactive glass has been investigated through micro-PIXE analysis, in connection with histological studies. Sol-gel-derived bioactive glasses doped with Sr were implanted in rabbit femoral condyle for 8 weeks. Micro-PIXE analyses were carried out on histological sections of the bone/bioactive glass interface. The study shows the Sr-doped bioactive glass grains being transformed into a new bone matrix, with a close contact with surrounding native bony tissues. Interestingly, traces of Sr ions are detected over a large area around the site of implantation, revealing that the Sr osteoinductive ions are efficiently released from the glass matrix and diffused over several ten microns through the surrounding native bony tissues. This micro-PIXE demonstration of the bioavailability of Sr ions suggests Sr-doped bioactive glasses should be favorably considered for enhanced *in situ* bone regeneration or related applications like treatment of osteoporosis.

Keywords: Bioactive glasses; Strontium; Osteoinduction; Osteoproduction

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Research Article

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Fabrication, Characterization and Drug Release of Ciprofloxacin loaded Porous Polyvinyl Alcohol/Bioactive Glass Scaffold for Controlled Drug Delivery

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Abstract

Scaffolds are implants used to deliver cells, drugs, and genes into the body in a local controlled release pattern which offers many advantages over systematic drug delivery. Composite scaffolds of polyvinyl alcohol (PVA) and quaternary bioactive glass (46S6 system) with different ratios of glass contents were prepared by lyophilisation technique. The broad spectrum antibiotic ciprofloxacin (Cip) was impregnated to the scaffold during the fabrication in a concentration of 5, 10 and 20%. Biodegradation rate and in-vitro mineralization of the prepared scaffolds were performed by soaking the scaffolds in simulated body fluid (SBF). Phase identification, microstructure, porosity, bioactivity, mechanical properties, intermolecular interaction and drug release pattern in PBS were characterized by XRD, SEM coupled with EDS, Hg-porosimeter, inductively coupled plasma-optical emission spectroscopy (ICP-OES), universal testing machine, fourier transform infrared (FTIR) and UV-spectrophotometer, respectively. A porous scaffold has been obtained with porosity up to 85%. By increasing the glass contents in the prepared scaffold the porosity and the degradation rate decrease however, the fracture toughness (K_{1c}) was enhanced. FTIR spectrums confirmed that the reaction mechanism between both the polymer and the drug is condensation reaction on both terminals of the Cip with the multi-chains of PVA polymer. A sustained drug release pattern was observed with a quasi-Fickian diffusion mechanism. The formulated ciprofloxacin loaded porous polyvinyl alcohol scaffold gave an acceptable physicochemical properties and was able to deliver the drug in a prolonged release pattern which offers a distinguish treatment for osteomyelitis as well as local antibacterial effect.

Keywords: Tissue engineering; scaffolds; Ciprofloxacin; Drug release; Freeze drying; Polyvinyl alcohol

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Research Article

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Preparation of Copper-Doped Hydroxyapatite with Varying X in the Composition $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y\text{H}_z$

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Abstract

Copper (Cu) ions are of interest with respect to materials for bone regeneration because of their angiogenic potential, which by stimulating infiltration of blood vessels into a bone substitute scaffold could increase the viability of bone forming cells within the scaffold and so hasten the healing process. Recently, the preparation of CuSO_4 -loaded calcium phosphate scaffolds and Cu-doped bioactive glasses for this purpose has been reported. [1,2]. Here we report the preparation of hydroxyapatite structurally doped with Cu ions.

Cu-doped hydroxyapatites with $x=0.01, 0.1, 0.2, 0.35, 0.5, 0.75$ and 1 in the nominal formula $\text{Ca}_{10}(\text{PO}_4)_6\text{Cu}_x\text{O}_y(\text{H})_z$ were prepared by solid state synthesis and analysed by powder X-ray diffraction (XRD) with Rietveld analysis of the data, Fourier transform infrared spectroscopy (FTIR) and diffuse reflectance UV-visible spectroscopy (UV-Vis). As x was increased, the lattice parameters increased, consistent with copper occupying hydroxyl sites in the hexagonal channels, rather than calcium sites. The c parameter increased more than the a parameter, correlating with the formation of Cu-O species oriented along the c axis, as proposed previously [3-5]. FTIR spectra confirmed the presence of Cu-O species on the OH sites, with a band at $771\text{-}778\text{ cm}^{-1}$ assigned to a Cu-O stretch (characteristic of Cu^+) in O-Cu-O units and with decreasing intensity of the OH stretch (3572 cm^{-1}) and the OH libration (631 cm^{-1}) bands as x was increased. Four bands, related to OH stretches disturbed by nearby Cu, were observed between 3140 and 3450 cm^{-1} . The intensity of these bands varied with increasing x , indicating changes in the OH environments as Cu-O species on the OH sites are progressively condensed to form O-Cu-O units and possibly $-(\text{O-Cu-O-Cu})-n$ chains. UV-Vis spectra showed three bands between 400 and 800 nm , assignable to d-d transitions of Cu^{2+} . Therefore we propose that Cu is present in these materials as both Cu^+ and Cu^{2+} .

Keywords: Hydroxyapatite; Copper; Solid state synthesis; XRD; IR; UV-visible spectroscopy

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Research Article

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In Vitro Assays: Comparative Study of Nanobioactive Glass System By Sol-Gel

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Abstract

Research in the field of bioactive glass is critical for enabling future breakthroughs in glass science and technology. This is referred to their ability to bond to bone and even to soft tissues. Different bioactive glass systems have been prepared by sol-gel. However, the production of Na₂O-containing bioactive glasses by sol-gel methods has proved to be difficult. The aim of this study was to prepare the quaternary system 46S6 of bioactive glass by modified sol gel techniques in aqueous solution under ambient conditions and study the structural transformations of it when treated at different temperatures. In addition, compare the behaviour of the prepared sol-gel bioactive glass system (SG-B) by its corresponding system prepared by melting technique (MB) before and after immersion in simulated body fluid (SBF). The obtained glasses were characterized by differential scanning calorimeters/thermogravimetric analysis DSC/TG, X-ray diffraction (XRD), X-ray fluorescence (XRF), Fourier transform infrared spectroscopy (FT-IR), transmission electron microscope (TEM), and scanning electron microscope (SEM). *In vitro* bioactivity test were also conducted in simulated body fluid (SBF). Furthermore, the effect of the bioactive glasses; MB and SG-B particles on the proliferation of Saos-2 cells was investigated. The results of XRF confirmed the preparation of 46S6 bioactive glass by sol-gel with 47.49% SiO₂, 24.8% CaO, 6.73% P₂O₅ and 20.58% Na₂O in molar percentage in few hours. Results of TEM show that the particles were not agglomerated with an average diameter less than 100 nm. The sol-gel derived nanoparticles revealed *in vitro* bioactivity both in SBF and over the Saos-2 cells higher than that prepared by melting technique. These results were shown to hold promising potential enabling their use as medical implantation materials and for future work elaboration for tissue engineering.

Keywords: Nanospheres; Bioactive glass; Sol-gel; Melting; 46S6; Heat treatment and bioactivity

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Research Article

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Influence of Surface Microstructure on Sub gingival Bacterial Adhesion Forces to Titanium and Zirconia Implant Materials

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Abstract

Peri-implantitis is one of the main causes of failure for oral implants. The bacterial adhesion and biofilm formation on the implant surface is the essential step and the prerequisite in the pathogenesis of the infection. The aim of this study was to investigate the subgingival bacterial adhesion forces to titanium and zirconia implant materials with different microstructure and surface roughness by atomic force microscopy (AFM). Titanium and zirconia specimens of different microstructure surface were obtained by polishing and sandblasting with the fine particles, which were divided into 4 groups according to surface roughness 10 nm, 100 nm, 200 nm, 300 nm. The surface morphology was observed by SEM and AFM. Water contact angles were measured on all specimens by the sessile drop technique. Adhesion forces between subgingival strains and surfaces were measured using AFM in nearly physiology conditions. Water contact angles were compared by ANOVA, while adhesion forces were analyzed by non-parametric statistics. Polishing and blasting produced the nanohole microstructure surface of titanium and zirconium with different roughness. With the increasing surface roughness, the contact angle increased and hydrophilicity was reduced. The surface hydrophilicity of titanium was lower than zirconia at the same roughness. For initially colonizing strains, the adhesion forces to zirconia increased from -0.09 nN to -0.87 nN while titanium increased from -0.28 nN to -0.96 nN with the roughness from 10 nm to 200 nm. When the roughness was more than 200 nm the forces decreased. For periodontal pathogenic strains, the adhesion forces to zirconia increase from -0.27 nN to -1.18 nN while titanium increased from -0.47 nN to -1.83 nN with the increasing roughness. The forces of periodontal pathogenic strains were stronger than initially colonizing strains while the forces to titanium surface were stronger than to zirconia. It is conclusion that zirconia can reduce bacterial adhesion force and the microstructure influences the subgingival bacterial adhesion.

Keywords: Bacterial adhesion forces; Surface microstructure; Subgingival strains; Titanium; Zirconia; Atomic force microscopy

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Research Article

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Effect of Sandblasting on the Mechanical Properties of Y-TZP Zirconia

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Abstract

Objective: This study aimed at evaluating relationship between mechanical property and morphology of surface roughness on tetragonal to monoclinic (t-m) phase transformation of zirconia that occurs after sandblasting using different ceramic abrasive materials. Testing hypothesis was that surface modification techniques affect phase transformation and morphology which in turn influences strength of zirconia biomaterials.

Methods: Zirconia plates (n=24) 10 mm width, 20 mm length, 1 mm thick were sandblasted using silicon carbide (SiC), alumina (Al₂O₃) and zirconia (ZrO₂) particles. Average surface roughness was measured by laser profilometer and morphology examined by scanning electron microscope (SEM). t-m phase transformation was identified by x-ray diffraction (XRD) patterns. Flexural strength of zirconia plates was determined by 3-point bending test. Load condition of fatigue test was determined by flexural strength. Weibull analysis was adopted to assess variability of strength. Fractured surfaces were observed by SEM to find fracture mechanism.

Results: SEM images of surfaces showed deep depressions with fine edges and intensity of this morphology varied accordingly with roughness value. Higher relative intensity of monoclinic phase in SiC blasted surfaces was identified by XRD patterns. Although there was no significant differences among the groups, SiC blasted surfaces showed slightly higher flexural strength, while Al₂O₃ blasted surfaces appeared stronger under load conditions. Weibull modulus of SiC blasted surfaces was higher indicating greater structural reliability. SEM observation of crack propagation revealed characteristic feature of fracture line process with branching and chipping.

Conclusion: t-m transformation ratio and flexural strength of Zirconia were largest after sandblasting by SiC abrasive material. However, fatigue strength was lower than that after sandblasting by Al₂O₃ which appears to be caused by surface morphology variation. Thus it can be concluded that sandblasting with SiC and Al₂O₃ has a marginally better impact on strength of the material as compared to ZrO₂.

Keywords: Zirconia; Sandblasting; Surface modification; XRD; Residual stress; Fatigue

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Research Article

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Restoration of Tooth Enamel Using a Flexible Hydroxyapatite Sheet Coated with Tricalcium Phosphate

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Abstract

Restoration and protection of tooth enamel are of great importance in operative and conservative dentistry. Hydroxyapatite (HAp) thin sheet can potentially be used for a novel dental biomaterial to repair the enamel. Using a pulsed laser deposition method and thin film isolation techniques, we have successfully created a freestanding flexible double-layered sheet composed of a HAp layer (4 μm in thickness) coated with a tricalcium phosphate (TCP) layer (500 nm). Our overall goal was to apply the newly developed HAp/TCP sheet to the restoration and protection of tooth enamel. In the present study, the adhesive characteristics of the HAp/TCP sheet on the enamel were evaluated mechanically and micro structurally, and were compared to those of mono-layered HAp sheet. The HAp and HAp/TCP sheets were adhered to extracted human teeth using calcium phosphate aqueous solution and artificial saliva. At 5 days after the adhesion, the mechanical properties of the sheet enamel interface were determined by tensile tests. By means of a stainless steel rod (3 mm in diameter) glued on the sheet with epoxy adhesive, tensile load was applied to the specimen until failure occurred. Furthermore, the interface structure between the sheet and enamel was observed by scanning electron microscopy. The adhesive strength (5.7 MPa) between the HAp/TCP sheet and enamel was markedly higher than that (1.9 MPa) between the HAp sheet and enamel. The electron microscopic observation revealed that the double-layered HAp/TCP sheet was widely fused with the enamel. The interfacial reaction layer in the HAp/TCP was thicker than that in the HAp sheet. These results indicate that the ultrathin layer of TCP on the HAp sheet has important roles in the mechanical integrity at the sheet-enamel interface. The double-layered HAp/TCP sheet can be used as a material to promote the repair of tooth eruption or to maintain healthy dentine.

Keywords: Hydroxyapatite; Tricalcium phosphate; Double-layered sheet; Pulsed laser deposition; Artificial enamel; Tooth remineralization

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Research Article

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Measurement of Piezoelectric Properties of Pulsed Laser Deposited Hydroxyapatite Thin Films on Platinum or Titanium Substrate

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Abstract

A new result which clearly showed the piezoelectricity of the hydroxyapatite (HAp) films was obtained in a laboratory experiment using the fabrication method based on the pulsed laser deposition (PLD) and the measurement of vibration response of the HAp structure. In order to measure the piezoelectric properties of the HAp films, we have fabricated Cu/HAp/Ti or Cu/HAp/Pt structure. At first, a 1.5 μm thick and 10 mm square HAp was deposited on a Ti or Pt substrate using the KrF PLD method, where the size of substrate was 80 mm long, 10 mm width and 0.3 mm thick. After the HAp deposition, the HAp film was crystallized by post-annealing at 350°C for 24 hours in nitrogen gas atmosphere and cooled slowly in an electric furnace. Then, a 70 μm thick Cu top electrode sheet was attached on the HAp film. Finally, one end of the Cu/HAp/Ti or Cu/HAp/Pt structure was clamped to compose a vibrating cantilever beam. Piezoelectric coefficients were estimated by output voltage responses of HAp films measured by a operational amplifier circuit when the Cu/HAp/Ti or Cu/HAp/Pt beam was excited by a shaker at the first natural frequency of the beam. Tip displacement responses of the beam were measured by a laser displacement sensor, and these results in conjunction with output voltage responses of the HAp were used to estimate the piezoelectric d_{31} coefficients of HAp films. It was verified that the HAp film possesses piezoelectricity, and further experiments were needed to establish the fabrication method of HAp films with excellent piezoelectric properties, which are currently ongoing projects.

Keywords: Hydroxyapatite; Piezoelectricity; Pulse laser deposition; Post-annealing; Vibration response; Cantilever beam

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Research Article

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Synthesis of Sulfate Ion-substituted Hydroxyapatite from Amorphous Calcium Phosphate

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Abstract

Introduction: The hydroxyapatite (HAp) composition is expressed by the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Various reports on synthesis of HAp with Ca ion substituted with various cations (Sr, Ba, etc.) have been published. On the other hand, reports on synthesis of anion-substituted HAp with PO_4 substituted with SO_4 or CO_3 ions have only been occasionally made, and reports on synthesis of sulfate ion-substituted hydroxyapatite (SAp) are rare. The present study investigates the synthesis conditions of SAp from amorphous calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$, ACP) as a starting material, which has the ability to readily take in various ions into its structure.

Experiment Method: Sodium sulfate (Na_2SO_4) was added to ACP or HAp so as to obtain the Na_2SO_4 /(ACP or HAp) weight ratio of 0-14. The sample was hydrothermally processed at 220°C for 3 h, and SAp was obtained after filtering, washing, and drying of the processed sample. For comparative data, the same procedure was also carried out using stoichiometric HAp as the starting material.

Results and Discussions: HAp was obtained as the product under all conditions, and Ca-deficient-type HAp was obtained from ACP. When HAp was used as the starting material, substitution by SO_4 was scarcely observed. However, when ACP was used as the starting material, the SO_4/PO_4 molar ratio increased with an increasing amount of added Na_2SO_4 . This result was observed up to a SO_4/PO_4 molar ratio of 0.5, meaning that 1/3 of the contained PO_4 in HAp was substituted with SO_4 . In addition, the result of FT-IR spectra showed that the HPO_4^{2-} absorbance decreased due to addition of Na_2SO_4 . Therefore, additive SO_4^{2-} substitution for HPO_4^{2-} within Ca-deficient-type HAp crystal structure was considered.

Keywords: Hydroxyapatite; Piezoelectricity; Pulse laser deposition; Post-annealing; Vibration response; Cantilever beam

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Research Article

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Characterization of β -TCP, β -TCMP and BCMP Produced by Hydrolysis

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Abstract

The overall objective of our study is to develop tricalcium phosphate (TCP), magnesium (Mg)-substituted TCP and Mg-substitute bicalcium phosphate (BCP) to produce 3D scaffolds for bone repair. The aim of this work was to obtain TCP, Mg-substitute TCP and Mg-substitute BCP by using the hydrolysis processing method and to evaluate their toxicity and their ability to promote cell differentiation. Calcium deficient apatites (CDA) were prepared by hydrolysis method. The CDA's were filtered and washed using approximately 6 L of double distilled water. The CDA's were calcined at 950°C for 11 h. The identification and compositions of the different CDA's before and after calcinations were confirmed using x-ray diffraction, FTIR and inductive coupled plasma. SEM images were obtained from the green and calcined powders. A colorimetric cell viability assay (MTT) was performed using a mouse osteoblastic cell line (MC3T3-E1) to determine the toxicity of the CDA preparations. Phenolphthalein test was executed according to the French standard NF S 94-066. A MC3T3-E1 cell differentiation test was performed to quantify nodule formation, as well as an enzyme-linked immunoassay (ELISA) to measure markers for bone reabsorption (TGF-Beta1 and collagen) and markers for bone formation (osteocalcin and alkaline phosphatase). β -TCP was obtained after calcining Mg-free CDA. β -TCMP or BCMP were obtained after calcining Mg-substituted CDAs, depending on the Mg/Ca molar ratio of the CDA. The MTT assay indicated cell proliferation for β -TCP, β -TCMP and BCMP, obtained from CDA's. The MC3T3-E1 cell differentiation showed a greater cell proliferation for the β -TCP, followed by the β -TCMP and BCMP. The same pattern was observed for the ELISA. The results from this study demonstrated that the hydrolysis processing method was effective to produce CDA's to obtain β -TCP, β -TCMP and BCMP. The calcium phosphates obtained from this method weren't toxic, promoted cell proliferation and have potential to promote bone formation.

Keywords: Tricalcium phosphate; Hydrolysis; Cell viability; Nodule formation; Bone markers

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Research Article

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Comparison of Spectroscopic Methods for Following Hydrolysis of Tc_p to Apatite

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Abstract

The hydrolysis of alpha tricalcium phosphate (α -TCP) impacts the reaction kinetics and the resulting injectability of minimally invasive cements, cohesion and mechanical properties. Real-time tracking will provide greater insight to the reaction kinetics and an important comparison with the evolution of mechanical properties. A range of spectroscopic methods have been chosen to determine the changes with hydrolysis of β -TCP and compared to X-ray diffraction. FTIR spectroscopy (DRIFT, ATR), Raman spectroscopy and X-ray diffraction were employed to for real-time assessment of the reaction kinetics. Nanosized β -TCP was chosen for a faster reaction time and a mixture with 5% β -TCP also included seeing the effect of a seed. Powders were mixed with water, flattened and the reaction followed over time. To minimize the exposure of irradiation, short analysis times were chosen with XRD over the range of 25°-35° and a different probing location was chosen with micro Raman spectroscopy. FTIR-ATR analyzed through the window thus ensuring constant water content in the reaction mixture.

Results reveal the advantages with each technique for quick analysis, ease of providing reproducible analysis conditions and the ability to identify changes during hydrolysis. X-ray diffraction showed good phase evolution and could detect early changes in the reaction. All spectra required further analysis by peak deconvolution with the easiest detection by micro Raman spectroscopy. Irradiation was found to remove water and prevent the reaction from reaching completion. The testing conditions are discussed in relation to larger β -TCP particles that react over a longer period of time and reproducible reaction conditions for a comparison between cements.

Keywords: Calcium phosphate cements; Tricalcium phosphate; Calcium deficient hydroxyapatite; Reaction kinetics; Raman and FTIR spectroscopy; X-ray diffraction

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Research Article

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NMR Spectra of Octacalcium Phosphate with Two Kinds of Dicarboxylic Acid Co-incorporated in Interlayers

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Abstract

Octacalcium phosphate (OCP: $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) well-known as a biomaterial has a layered structure composed of apatitic and hydrated layers. Dicarboxylate ions can be incorporated in the OCP structure. Investigation of OCP carboxylate (OCPC) could provide novel functional materials because chemical and physical properties of OCPCs depend on dicarboxylate ions incorporated into OCP. Though previous researchers focused on synthesis of OCP incorporated with one kind of dicarboxylate ion, recently OCPs incorporated with two kinds of dicarboxylate ions have been successfully synthesized.

However, the structure, such as how to incorporate two kinds of dicarboxylate ions, is not yet reported. The purpose of this study is to investigate the structure of OCP with incorporation of two kinds of dicarboxylic acids by observation of the local structure in OCPC through NMR measurement. OCP without dicarboxylate ions and seven kinds of OCPCs with doped succinic acid (Suc; $\text{HOOC}(\text{CH}_2)_2\text{COOH}$) and/or suberic acid (Sub; $\text{HOOC}(\text{CH}_2)_6\text{COOH}$) are used in this study. OCPCs were synthesized using ultrapure water including Suc and Sub with various molar ratios. Hereafter, the notation of the OCPC samples is denoted as (100-X) suc-X sub, where X indicates the molar ratio of Sub added to the ultrapure water.

According to ^{31}P NMR spectra, total peak intensity of the fifth phosphorus (P5) and the sixth phosphorus (P6) located in the hydrated layer of OCPCs is smaller than that of undoped OCP. This suggests HPO_4^{2-} ions including P5 and P6 located in the hydrated layer are replaced by incorporated dicarboxylate ions. In the ^{13}C NMR spectra, there are two peaks around 180 ppm related to carboxyl group in OCPC structure for the samples of X=0 and 100, while only one peak of carboxyl group is present in pure Suc and Sub as starting materials. This indicates there are two molecular orientations of carboxyl groups existing in OCPCs.

Keywords: NMR; Octacalcium phosphate (OCP); Carboxylate

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Research Article

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Effects of Cu, Zn and Ag on the Chemical Reactivity and Morphology of Doped Bioactive Glasses

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Abstract

Belonging to the family of biomaterials ceramics, bioactive glasses present a great interest due to their interesting properties for bone filling in the orthopedic, maxillofacial and dental surgery. When they are placed in contact with living biological tissues, bioactive glasses cause a list of physical and chemical reactions which involve the formation of hydroxyapatite crystals.

In this work, glasses were prepared by fusion technique and doped with different chemical elements such as Cu, Zn and Ag. These elements are present in the bone tissue and have physiological interests. Indeed, zinc is involved in various biological processes such as enzymatic reactions, the proliferation of osteoblasts and the synthesis of proteins. Copper and silver are particularly interesting elements for their antibacterial, anti-inflammatory and anti-infectious properties.

The obtained results show the formation of a hydroxyapatite layer $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ when the bioactive glasses are immersed in a simulated body fluid (SBF).

Using ICP-OES (Induced Coupled Plasma-Optical Emission Spectroscopy), we measured the calcium, phosphorus, silicon and doping elements concentrations to understand the kinetic of bioactivity and to have the kinetic of release of each doped glasses. The results showed after 30 days of immersion that Ag is continually released to reach 1.4 ppm; copper concentration reaches 0.38 ppm and Zn element keeps a low concentration 0.032 ppm.

Using SEM, the results showed that doping elements according to their content slow down the bioactivity and change the morphology and the relief of the apatite crystals which forms on the glass surfaces. The cytotoxicity assays shows that cells survive when they are in contact with our doped glasses.

Keywords: Copper; Silver; Zinc; Bioactive glasses; Chemical reactivity; Cytotoxicity

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Research Article

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Synthesis of Chromium Substituted Hydroxyapatites

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Abstract

Apatite in bone contains metal ions and the apparent lack of complications suggests that the body can accommodate foreign cations. Hydroxyapatite (HAp) can accommodate different cations such as strontium (Sr), iron (Fe), zinc (Zn) and chromium (Cr). Substituted cations affect the processability and the resulting biological behaviour. Chromium modified apatites have received very little attention to date.

Chromium is an important component in biochemical processes, and it's also found in bones. This provides the rationale to investigate chromium substituted apatites for bone repair. The daily allowable chromium intake is 35-45 µg. Unfortunately Cr (VI) is toxic and carcinogenic, but Cr (III) ions are not considered as a health hazard. Other oxidation states include Cr (IV) and Cr (V).

This work involves the synthesis of four chromium substituted apatites from a low crystallinity precursor. Synthesis of apatites was conducted so as to replace calcium ions with chromium (III) ions at different molar contents (1%, 3%, 5%, 10%). Thermal stability was determined and the crystallized calcium phosphate analyzed by qualitative phase analysis and quantitative elemental analysis. Thermal stability of hydroxyapatites was determined by differential thermal analysis and thermogravimetry. X-ray photoelectron spectroscopy explored the oxidation state of Cr in the low crystallinity precursor and the crystallized apatite.

Higher concentrations of chromium increased the crystallization temperature. Calcium ions replaced chromium at less than the theoretically expected value. The inclusion of Cr leads to a decrease in the crystallinity pointing to a lower structural order. Concentrations up to about 5% could be included, but at 10% substitution, the apatite structure was no longer retained. XPS studies revealed an oxidation state of Cr (IV) in the crystallized Cr apatites.

Chromium modified hydroxyapatite after crystallization at 650°C showing incorporation up to 5% Cr.

Keywords: Chromium; Hydroxyapatite; Characterization; Synthesis

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Research Article

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Effect of Hydroxyapatite on Reaction of Dicalcium Phosphate Dihydrate (DCPD) and Fluoride Ion

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Abstract

DCPD, dicalciumphosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) reacts with fluoride ion in an aqueous solution, and forms fluorapatite (FAP, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$). This reaction founded in reaction of dental caries prevention. We have applied the reaction to stabilization of fluoride and various heavy metal ions in the environments such as waste water, polluted ground water, and polluted soil. We found that the reaction of DCPD with small amount of fluoride ion do not carry on directly. By mixing DCPD with water, nano-scale precursor particle forms on surface of the DCPD particle. The formation of the precursor particle needs few hours of induction period. Such induction period (lag time) is problem for application the reaction to the environmental issues. In previous studies, we found that phase of the precursor particle seems to be hydroxyapatite (HA) like calcium phosphate. In this presentation, effect of HA on reaction of DCPD with fluoride ion was investigated. Change of morphology of DCPD was studied. Particle morphology did not change before and after reaction of single phase of DCPD. Mixing HA in DCPD was appeared improvement the lag time, however changed particle morphology of DCPD into morphology of HA. We carried on coating of HA on the DCPD particle by soaking DCPD in simulated body fluid (SBF, Kokubo Solution). It was appeared that HA coated DCPD reacted with fluoride ion, and particle morphology of formed FAP did not change from DCPD morphology. These results suggest that shape and particle size of FAP after reaction of DCPD is controllable by control of shape of DCPD particle and coating of HA. In stabilization of fluoride ion in the environment by forming FAP, separating of formed FAP is one of the problems of application. From the results, coating of HA on DCPD particle is applicable to treatment of fluoride in the environments.

Keywords: DCPD; Fluoride; Transform reaction; HA (hydroxyapatite); FAP (Fluorapatite); Hybridization

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Research Article

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Injectability and Cohesion of α -Tricalcium Phosphate Based Cements

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Abstract

Injectability and cohesion are interlinked properties of calcium phosphate cements. It is expected that, if the cement paste has higher solid phase/liquid phase ratio, the cement will be less injectable, but the cohesion of the cement will improve. In this work the possibility to improve injectability of α -tricalcium phosphate (α -TCP) bone cements using citrate ion containing additives was investigated. It has been ascertained previously that composition of setting solution can significantly alter the setting process. It was evaluated how the presence of citrate ions affected injectability and cohesion for cements with different compositions of liquid phase.

It was ascertained that the cohesion of calcium phosphate cement was strongly correlated with setting time and that the presence of citrate ions was not a significant factor (cement compositions with similar setting times were compared). The injectability was higher for cements with basic liquid phase pH (>7) and cements could be injected completely. If cement has acidic liquid phase pH (<7), injectability is low, because of the initial precipitation of calcium hydrogen phosphate dihydrate (brushite). Presence of citrate ions prevents precipitation of brushite, but also decreases the setting time significantly. Thus while the presence of citrate ions can affect setting of cements based on α -TCP, it is not possible to improve injectability in a significant manner.

Keywords: Calcium phosphate cements; Bone tissue regeneration; Calcium phosphates; Cohesion; Injectability

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Research Article

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Improvement of Radiopacity for Injectable Bioceramics

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Abstract

One of the main difficulties encountered by the surgeon using resorbable injectable bone substitute materials based on calcium phosphate (CaP) is the difficulty of controlling the successful implantation immediately after the operation. Self hardening calcium phosphate cement as MCPCTM, or non self hardening putties as MBCP gelTM (mixture of BCP granules and hydrogel) display indeed similar radioopacity as autologous bone which can lead to issues during the surgery or x-ray controls. It would therefore be desirable to include contrast agents used in bone replacement surgery.

The obstacles encountered in this area are, firstly, the difficulty of obtaining a homogeneous distribution of the contrast agent within the CaP matrix, and secondly the toxicity of existing contrast agents, or the decrease of the bioactivity and biological properties as osteoconduction, osteostimulation, ionic absorption of the CaP bioceramics for bone regeneration. Many of contrast agents, such as BaSO₄ or ZrO₂ have significant side effects and can induce pathological bone resorption.

The purpose of this paper was to demonstrate the biocompatibility of an innovation in improving radiopacity of CaP bioceramics. We have obtained by chemical synthesis and sintering a bone substitute consisting of β -tricalcium phosphate and hydroxyapatite combined with a contrast agent for medical imaging, which induces good bone growth without being cytotoxic. The contrast agent was integrated in the CaP apatite by substitution of some calcium by barium ions. The granular composition for biomaterials tested, β -tricalcium phosphate and hydroxyapatite, containing HA substituted with Barium. The granules were associated to the self setting calcium phosphate MCPC (Biomatlante France), the HA containing Barium was uniformly distributed in the mass of the composition.

Nine rabbits were implanted during 6, 12 and 24 weeks according ISO 10-993 with control samples (standard MCPC) or with improved radiopacity samples (MCPC RO). Organs histopathological analyses were realized.

No cytotoxicity was observed, both in bony site, and in the 5 organs analyzed.

	6 weeks		12 weeks		24 weeks	
	MCPC	MCPC RO	MCPC	MCPC RO	MCPC	MCPC RO
Newly formed bone	12%	16%	18%	23%	16%	21%
Residual biomaterials	61%	52%	29%	29%	40%	32%

No statistical difference was measured on bone in growth and resorption between the control and the MCPC RO.

Keywords: Calcium phosphate cements; Bone tissue regeneration; Calcium phosphates; Cohesion; Injectability

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Research Article

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Preparation and Characterization of Magnesium-Substituted Calcium Phosphate Bioceramics

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Abstract

Osteoporosis is a progressive and debilitating metabolic bone disease characterized by bone loss and structural deterioration leading to increased bone fragility and susceptibility to fractures. On the cellular level, magnesium ions are reported to stimulate osteoblasts proliferation. Magnesium containing calcium phosphate biomaterials promote the formation of bone and could prevent and treat osteoporosis and repair the fractures caused by osteoporosis and other diseases. Of great interest in developing artificial bone is the incorporation of magnesium ions into the calcium phosphate ceramics lattice in order to improve the physico-chemical and structural properties of the material. The objective of this work is to prepare magnesium-substituted hydroxyapatite, β -tricalcium phosphate and biphasic calcium phosphate bioceramics with magnesium concentration closebone-like amounts of magnesium and investigate the effect of magnesium incorporation into different calcium phosphate structures on the properties of synthesized and sintered calcium phosphate biomaterials. This work reports a systematic investigation on magnesium-substituted calcium phosphates. Magnesium-substituted calcium phosphates were prepared by the modified wet chemical precipitation method – precipitation from an aqueous medium by slow addition of H_3PO_4 solution to a mixture of $Ca(OH)_2$ and $Mg(OH)_2$. As-synthesized and thermally treated calcium phosphates were investigated using different methods and techniques, including Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), differential thermal analysis (DTA) and field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDS) and N_2 adsorption using Brunauer–Emmett–Teller (BET) method.

Keywords: Magnesium-substituted; Calcium phosphates; Bioceramics; Wet synthesis

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Research Article

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Bone Regeneration using Mesenchymal Stem Cell and Biomaterials - The Reborne Experience

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Abstract

Bone is the most transplanted tissue in human with about 1 million procedures annually in Europe. Autologous bone graft is the gold standard in bone regeneration but it requires a second surgery, is limited in quantity and often associated with complications. Synthetic calcium phosphate biomaterial in association with mesenchymal stem cells is a potent alternative to autologous bone grafting. Starting from a bone marrow aspirate, several hundred millions of mesenchymal stem cells are produced in 3 weeks in culture medium containing human blood platelet lysate. These cells are fixed on calcium phosphate granules and then implanted in subcutis of nude mice where they produced mature bone tissue. The mixture of human mesenchymal stem cells and biomaterial is also effective in bone healing of critical size defects in calvarias and femurs of nude rats. The procedure has also proven efficacy in regenerating diaphysis defects in metatarsi of sheep. The European project REBORNE aims at bone regeneration at the clinical level in non union fractures, treatment of osteonecrosis of the femoral head as well as jaw augmentation prior to dental implants and reconstruction of cleft palates in children. This presentation will give the latest pre clinical results that have led to the authorization by medicinal agencies to translate this regenerative medicine technology to patients.

Keywords: Bone tissue engineering; Mesenchymal stem cells; Biphasic calcium phosphate

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Research Article

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Development of Gene Activated Matrices for Tissue Regeneration in Osteoarthritis

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Abstract

Osteoarthritis is a degenerative disease of the joints that comprises degeneration of cartilage and underlying bone as well as inflammation. Due to unknown etiology treatment is mainly symptomatic ranging from pain reduction and anti-inflammatory drugs to ultimately joint replacement.

The aim of the EU-funded project GAMBA is to develop a gene activated matrix (GAM) concept that allows for spatio-temporal control of growth factor expression involved in cartilage and bone regeneration. GAMs are biomaterial scaffolds comprising gene vectors. Cells growing on or into the matrix will become infected/transfected by the immobilized or released vector and will consequently express the growth factor genes, resulting in local autocrine and paracrine stimulation of a desired differentiation process.

Resorbable bone substitute material (MBCPTM) and a thermo-reversible hyaluronan based hydrogel (HA-gel) are used as scaffolds. Bone marrow-derived mesenchymal stem cells are incorporated into scaffolds together with different gene vectors coding for reporter genes or growth factors involved in cartilage and bone regeneration (BMP-2 and TGFβ) and an anti-inflammatory gene (vIL-10). Temporal control of gene expression is achieved using the Tet-on system.

Spatial control of gene expression was achieved by differential loading of gene vectors expressing reporter genes in 3D culture. Temporal control was demonstrated by doxycycline induction of growth factor release in 2D and 3D culture.

Spatial and temporal control using MBCPTM and the HA-gel as scaffolds was achieved using non-viral and adenoviral vectors. Our results indicate that the concept of GAM might be feasible for regulated growth factor expression upon command and demand. Analysis of gene expression induced differentiation of MSC is ongoing.

The scientific and technological objectives of the GAMBA project are complemented with an innovative program of public outreach, actively linking patients and society to the evolvement of this project. GAMBA is funded by 7th Framework Programme of the EU, grant number NMP3-SL-2010-245993.

Keywords: Gene therapy; Gene activated matrices; Adenoviral vectors; Non-viral vectors; Mesenchymal stem cells

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Research Article

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Osteoconduction, Osteogenicity, Osteoinduction, What are the Fundamental Properties for a Smart Bone Substitutes

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Abstract

Resorbable synthetic bone graft materials are mainly calcium phosphates. These materials differ in chemical composition and physical properties, particularly in regards of osteoconduction, osteogenic and/or osteoinductive properties. Several scaffolds are characterized and compared. Results from preclinical and clinical studies are selected. Osteoconductive properties have been largely described but osteoinductive properties have been less explored and documented. The purpose of this lecture will be to present series of data demonstrating the differences in scaffolds for bone regeneration and to explain how dissolution and, biological precipitation into the micropores occur simultaneously with osteoid and bone formation after implantation in bony sites and non bony sites and support the osteogenic/osteoinductive properties.

Smart scaffolds for bone regeneration must integrate: chemistry, physic chemical surface properties, micro and macro structure.

Two large European programs of the 7th PCRD are investigating Bone Tissue Engineering (REBORNE) and Gene Osteoarticular Therapy (GAMBA) using Smart Bone Scaffolds based on these optimal properties.

Keywords: Bioceramics; Osteoconduction; Osteogenicity; Osteoinduction

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Research Article

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Calcium Phosphates Granules Development for Self-setting Injectable Bioceramics

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Abstract

The need for Minimally Invasive Surgery (MIS) prompted the development of self-setting injectable, mouldable calcium phosphate bone void filler (CPC) as bone substitutes. Currently, several calcium phosphate bone cements are commercially available and more are being investigated.

The concept was first introduced by Le Geros et al. in 1982 and the first patent was obtained by Brown and Chow in 1986. All the current CPCs are reported to have good mechanical properties and reasonable setting times. However, after setting, these materials remain dense and do not provide rapid bone substitution.

On the other side, Biphasic Calcium Phosphate (BCP) granule is a bioactive concept based on mixture of Hydroxyapatite (HA) and Beta-Tri Calcium Phosphate (β -TCP). The concept is determined by an optimum balance of the more stable phase HA and more soluble TCP. The material is soluble and gradually dissolves in the body, seeding new bone formation as it releases calcium and phosphate ions into the biological medium, involving high osteoconductive and osteogenic properties. The self-setting calcium phosphate bone void filler (MCPCTM, NYU, Nantes University and Inserm) was the combination of microporous BCP granules and a setting matrix easily absorbed after implantation. Residual granules of bioceramics acting as smart scaffolds for bone in growth and bone regeneration at the expense of the calcium phosphate cement. Preclinical studies, in small and large animals, human clinical cases, demonstrate the performance and efficacy of this concept of calcium phosphate cement. MBP granules act as a scaffold for bone osteo conduction, and resorption of the amorphous calcium phosphate (ACP) content of the cement allowed macro porosity and bone in growth between and at the surface of the BCP granules, extending to the core of the implanted site.

Keywords: Bioceramics; Calcium phosphate cement; biphasic calcium phosphate

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Research Article

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Comparative Critical Study of Commercial Calcium Phosphate Bone Substitutes in Terms of Physico-chemical Properties

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Abstract

The physico-chemical characteristics impact directly or indirectly the bioactive properties of biomaterials, it is then essential to correlate it with their effect *in vivo*. A panel of biomaterials available on the market, based on Hydroxyapatite (HA) and Tricalcium phosphate (β -TCP) is studied in terms of surface area, hydrophilicity, porosity, zeta potential, crystalline phases and density. This study highlights the dispersity of commercial calcium phosphates (CaP) properties, and demonstrates how the quality criteria required for such bone substitute based on biomimicry concept, whose pores distribution is certainly the more relevant, are often incompletely or not respected according to literature.

Keywords: Calcium phosphate; Commercial biomaterials; Pores distribution; Bone substitutes

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Research Article

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Osteoinduction of Biphasic Calcium Phosphate Granules in Critical Size Defects After Osteonecrosis Induced by Focal Heating Insults

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Abstract

A simple, rapid and inexpensive induction method of osteonecrosis by heat (80°C), in rabbit femoral defect model, was assessed with or without BCP granules (MBCP+®, Biomatlante SA). The osteonecrosis model has been validated after highlighting significant statistical differences between positive control and osteonecrosis sites ($p < 0.001$) in terms of bone remodeling. The osteoinductive effect of BCP granules has been proven statistically ($p < 0.05$) after image analysis of new bone presence in osteonecrosed empty sites and osteonecrosed sites containing BCP granules from 6 weeks.

Keywords: Osteonecrosis; Biphasic calcium phosphate; Osteoinduction; Image analysis; *In vivo*

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Research Article

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Composite Bioceramics/Polymer Electro Spun Scaffolds for Bone Tissue Engineering

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Abstract

The need of both bioresorbable and osteoconductive 3D scaffolds able to recruit stem cells and enhance their differentiation to new bone, lead to innovative composite biomaterials synthesis for bone tissue engineering and gene therapy. In the context of GAMBA 7th PCRD european project, the main goal of this study was to succeed in the relevant association of well-known osteoconductive biphasic calcium phosphate (BCP) made of Hydroxyapatite (20% HA) and β -Tricalcium Phosphate (80% β -TCP) crystallographic phases and resorbable poly(L-lactide-co-D,L-lactide) (PLDLLA) 3D matrices synthesized by electrospinning. Two types of composites were used, one with BCP granules, and the other with BCP microparticles. It appeared BCP granules/PLDLLA composite 3D matrices allowed high cell adhesion, and higher stem cell differentiation in vitro thanks to their specifically engineered physico-chemical properties and are promising scaffolds in terms of cells or active ingredients carrying.

Keywords: Fibers; Biphasic calcium phosphate; Composite; Scaffold; Cell adhesion

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Research Article

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Physical Properties and *In vitro* Tested Biological Performance of Bioactive Glasses and Glass-Ceramics

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Abstract

Bioactive glasses prepared by both melt quenching and sol-gel methods were precursors of bioactive glass-ceramics. The structure of the samples was modified by calcination under different conditions. After incubation of the samples in simulated body fluid, on their surface self-assembled an apatite type layer in vitreous or nanostructured state, depending on the sample composition and structure, and on immersion regime. The structure of the samples and the distribution of the structural units was investigated by X-ray diffraction, infrared spectroscopy and nuclear magnetic resonance. Changes of atomic environments were followed also by X-ray photoelectron spectroscopy. Human osteosarcoma cell line (MG63) was used for the *in vitro* cellular response. DNA staining (Hoechst 33258) assay was performed for assessing samples colonization.

The shifts recorded for XPS binding energies depend on heat treatment parameters and point out changes in the covalency degree of calcium and phosphorus bonds with oxygen atoms, and this interferes with samples bioactivity. The nanosized crystalline phases developed by calcination favour the self-assembling of a hydroxyapatite type layer in simulated body fluid and enhance the cells function of the cells adhered onto samples.

Keywords: Bioactive glasses; Glass-ceramics; XRD; FTIR; XPS; *In vitro* cellular response

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Research Article

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Bioceramics Influence on the Biodegradability of Collagen Based Composite for Hard Tissue Regeneration

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Abstract

Bioceramics are produced in many forms and could present a variety of phases, with different functions in repair of the human body. In many biomedical applications bioceramics are used in the form of bulk or porous materials but in some cases the bioceramics are used in powder form in a composite material with polymeric matrix, in order to achieve the enhanced mechanical and biological properties. In the case of composite materials with polymeric matrix, not just the chemical composition of bioceramics used as reinforcement component have an influence on the composite properties, but also the microstructural aspects of bioceramics such as homogeneity, phase distribution, morphology, grain size/grain shape, and surface, etc.

In this paper, we will present the effect of the bioceramics type (tricalcium phosphate, hydroxiapatite), ratio and structure on composite material structure, the biodegradation properties and cytotoxicity of some collage based composites obtaining using the freeze-drying process. Also, a comparative analysis between this experimental composites and other collagen based composite reinforced with magnesium powder will be made in order to show the positive effect of bioceramics in this type of composite materials. The experimental biocomposites were prepared by freeze-drying for 48 h of collagen gels having the different weight ratio (1%, 3% and 5% wt) of magnesium powder, hydroxiapatite powder, β -tricalcium phosphate and a mixture Mg+10% β -TCP, and cross-linked with glutaraldehyde. In order to find the appropriate properties of the composites for using in the human body we have perform different measurements: porosity, water absorption, in vitro degradation test using collagenase as medium, biocompatibility testing.

Keywords: Composite materials; β -Tricalcium phosphate; Hydroxyapatite; Collagen; Freeze-drying; biodegradation

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Research Article

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Si-HPMC Hydrogel Reinforcement using Particles

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Abstract

Tissue engineering is a multidisciplinary field combining chemical, engineering and life science expertises in order to develop functional structures for damage tissues. Cells are generally seeded into the extracellular matrix (ECM). These biomaterials are prepared as a temporary scaffold to improve mass transfer, support cell growth and enhance the cells to produce molecules they are supposed to. Until recently, it was believed that biomaterial selection was performed based on its macroporous, biocompatibility and degradability properties and its ability to withstand biomechanical stress. Engineering and material issues are now under focus to develop suitable scaffolds for tissue engineering. Indeed, Discher et al. have shown that material stiffness induces critical effects on cell behavior and differentiation. Therefore, LIOAD has been working on the development of hydrogels as ECM for tissue engineering (e.g. bone and cartilage regeneration). They prepared a cellulose based polymer capable of self cross-linkage (Si-HPMC) to avoid any toxicity issues resulting from using cross-linking chemicals or photo cross-linking. The objectives of the present work are to modulate and study the stiffness of Si-HPMC hydrogels by adding nano (laponites, clay based particles) reinforcement.

The results show that we managed to modulate the stiffness of Si-HPMC hydrogels by adding Laponites nanoparticles. Confocal micrographs analysis has demonstrated that the protocol used to incorporate laponite particles within the hydrogels has a strong influence on the 3D structure morphology. Laponites appear to induce the formation of a dual network (due to the self gelation properties of laponites in water). This dual network formation can be correlated to the 10 times increase of the G' modulus.

Results prove that we can modulate the stiffness of hydrogels which should be very powerful for tissue engineering applications because of the increase of their strength to support biomechanical stress.

Keywords: Hydrogel; Tissue engineering; Reinforcement; Dual network

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Research Article

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A New Synthetic Diet Increased Bone Mineral Density and Strength of Ovariectomized Rat

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Abstract

A synthetic diet consists of magnesium (Mg), zinc (Zn) and fluoride (F) ions incorporated in a carbonate apatite. This study aimed to determine changes in bone mineral density (BMD) and strength induced by the synthetic diet. Eight ovariectomized female Wistar rats (OVX) (20 weeks old) were used in this study. OVX were divided into two groups (n = 4): Group 1 fed the normal mineral diet (NMD), and Group 2 fed the synthetic diet (SD: 0.74% Ca, 0.48% P, 0.10% Mg, 365.4 ppm Zn, 51.01 ppm F and 0.27% CO₃). The rats were sacrificed after a lapse of eight weeks and femur isolated and cleaned of soft tissues. The BMD distribution of the femur mid-shaft was evaluated to assess bone quality using micro-computed tomography, using three-dimensional pseudo-color maps (3D-map). Bone strength was measured by a 3-point bending test. The results showed that the values of BMD for SD were higher than those of NMD. In the 3D-map of NMD, the BMD of the exterior of the cortical bone was high, and that of the region adjacent to the marrow cavity side was moderate. In contrast, the BMD of the exterior was high but the marrow cavity side region was mainly high but partly moderate for SD. And the bone strength values of SD were significantly higher than those of NMD. These results indicated that SD stimulated bone quality, increased BMD and bone strength. SD has potential to prevent bone loss and for osteoporosis therapy.

This research was started in collaboration with Prof. Racquel Z. LeGeros.

Keywords: Carbonate apatite; Ovariectomized rat; Bone mineral density; Bone strength; Micro-computer tomography; Synthetic diet

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Research Article

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Contribution to the Development of a Composite Biomaterial for Maxillofacial Bone Tissue Engineering

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Abstract

The main alternative to autologous bone graft for the reconstruction of hypotrophic bone consists in biomaterial-assisted mesenchymal stem cells (MSC)-based regenerative medicine.

To limit the massive cell death of MSC encountered after implantation, notably caused by ischemic environment or host proinflammatory T cells, we developed hydrogel and/or calcium phosphate, loaded with glucose and/or anti-inflammatory substance (acetylsalicylic acid ASA) as scaffold for MSCs.

The aim of this study was to determine the encapsulation/release profiles of the loaded molecules and physicochemical characterization of these loaded scaffolds.

Materials and Methods: Micro- macroporous Biphasic Calcium Phosphate (MBCP+) granules and/or Si-HPMC hydrogel associated with glucose 5 g/L (as anti-ischemic molecule), or acetylsalicylic acid 200 mg/L (as non steroidal anti-inflammatory drugs). Release profile and physicochemical properties (G', G'') were evaluated using UV spectrophotometer and rheometer.

Results: As results, MBCP+ granules have shown a flash release of the loaded drugs. However, Si-HPMC loaded with glucose, have reached a release of about 60% and 53% after 32 days in H₂O and PBS respectively. The influence of the loading has been evaluated under shear stress and the addition of the two molecules has induced opposite effects on Si-HPMC hydrogels. Indeed, while ASA showed no influences, glucose decreases the storage modulus.

Conclusion: These preliminary results, in particular the release profile of nutrients and mechanical behaviour could suggest interactions between the loaded molecules and the hydrogel network. As further investigations, the in vitro and in vivo biological effects of these new composite scaffolds will be paid attention.

Keywords: Bone tissue engineering; Calcium phosphate ceramic; Hydrogel; stem cells; Glucose; Acetylsalicylic acid

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Research Article

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Surgical Technology for Mandibular Radio Necrosis Reconstruction using Calcium Phosphate Bioceramics and Total Bone Marrow

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Abstract

Treatment of oral carcinomas usually requires surgical removal followed by radiotherapy. Since tumors are frequently bulky, bone is often involved and surgical procedure requires large resection. Free flaps still constitute the gold standard in mandibular reconstruction. Complications lead to the necessity to investigate alternative therapies. Since bone graft and bioceramics alone have failed, adjunction of osteoinductive properties has been investigated. Total bone marrow graft use has been investigated in animal and in human clinical application. We have recently proposed the concept of an immediate post-radiation bone marrow graft to provide an osteoinductive potential to the ceramic with good results. According to these data, a pilot study promoted by Nantes and Rennes Hospital ENT department was in progress in human for bone tissue engineering approach to patients presenting mandible cancer-related bone defects.

Methods: Animal experiments were realized in the Nantes National Veterinary School (Oniris), according to European directive number 86/609/CE. The study was conducted in accordance with animal experiment ethics rules. Two cm segmental defects were created in femoral diaphysis of Zealand rabbits, and femoral critical sized defect in a canine model.

Clinical study: Curative technology of radio necrotic mandibular bone defect. Multicentric study, Nantes Hospital promotion (ID RCB: n°2008-A01593-52; Ref BRD 08/10-H). Clinical trial phase 1, non-randomized, Bicentric Nantes-Rennes, Ten patients were involved with Osteoradionecrosis with surgical ablation, and basilar mandibular cortical preserved.

Results: Successful osseous colonization was found suggesting an osteogenic role of the bone marrow graft in the center of the defect after radiotherapy. According to these data, the pilot study promoted by Nantes and Rennes Hospital ENT department was in progress in human for bone tissue engineering approach to patients presenting mandible cancer-related bone defects. Clinical follow up revealed no adverse effects and architected bone regeneration. Some patients showed limited side effect related to insufficient soft tissue reconstruction. In spite of the newly formed bone exposure, the bone regeneration was maintained.

Conclusions: This pilot study demonstrated the efficiency of total bone marrow combined to synthetic bone graft (MBCP+®) for bone regeneration in low trophic area, particularly for radio necrotic bone defects.

Our findings demonstrate that a micro macro porous CaP bioceramics bone graft followed by a post-radiation can be revitalized using total autologous bone marrow graft. This has significant implications for bone tissue engineering approach to patients with cancer-related segmental bone defects.

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Research Article

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Novel Doxycycline Eluting Calcium Phosphate Foams

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Abstract

Calcium Phosphate Cements (CPCs) have been widely investigated as local drug delivery systems as an added value to their high potential for bone regeneration. CPCs are intrinsically nano- and microporous, but this is sometimes insufficient to allow adequate fluid exchange in the bulk of the matrix, restraining the complete drug release. Introduction of macropores in CPCs emerges as an interesting alternative.

Calcium Phosphate Foams (CPF) are the macroporous counterparts of CPCs, which maintain their characteristic features while adding the benefits of open macroporosity in views of cell colonization and thus, bone regeneration. This macroporosity can improve the fluid flow through the material, and thus, will most probably strongly modify the drug release kinetics.

It is the aim of this work to develop and characterize antibiotic-eluting self-setting CPF. To this end, a widely-used antibiotic in periodontal diseases (Doxycycline hyclate) is added to the solid phase of the cement (α -Tricalcium Phosphate), which is then blended with the foamed liquid phase, which is used as template for obtaining of a solid hydroxyapatite foam. The effect of the antibiotic addition on the setting reaction and structural parameters of the foam is studied, and the antibiotic release profile is determined for different antibiotic concentrations. Different amounts of the antibiotic lead to important modifications in the Calcium Phosphate foam architecture, such as increased pore interconnectivity, opening interesting prospects for the materials. Results show that calcium phosphate foams are able to release higher drug percentages (36% at 100 h) than their unfoamed counterparts (2.5%), and the antibiotic release is sustained for longer periods of time.

Keywords: Calcium phosphate cement; Antibiotic; Doxycycline

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Research Article

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BMP-2 Release from Low-Temperature Calcium Phosphate Foams

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Abstract

It is nowadays well acknowledged that the success of a biomaterial loaded with growth factors (GF) will only be achieved with an adequate release of the GF at the site. With this regards, the present work aims to determine the *in vitro* release profile and the biological activity of the BMP-2 (bone morphogenetic protein 2) loaded on calcium phosphate (CaP) foams of different compositions. To prove the osteoinductive capacity of the various materials, subcutaneous implantations in athymic nude mice have been made. Foams with different compositions were obtained by foaming alpha-tricalcium phosphate (α -TCP) slurries and allowing their setting for specific times. This yielded foams with a controlled content of α -TCP and hydroxyapatite (HA) of 50, 80 and 100% of HA respectively. *In vitro* results showed that the BMP-2 released from the foams and its activity, as determined by osteocalcin production in human mesenchymal stem cell cultures, depended on the composition of the foams. The foam containing 80% of HA showed the most sustained released and the highest activity for the BMP-2. The 8 weeks-ectopic implantation of the foams corroborated this trend as shown by the significant increase in bone neoformation, bone marrow and blood vessels formation. In conclusion, the composition of the CaP substrates strongly influenced the protein release pattern, which in turn resulted in a different *in vivo* performance of the BMP-2 loaded scaffolds in terms of osteoinduction potential.

Keywords: Calcium phosphate foam; BMP-2; Osteoinduction; *In vivo*; Release

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Research Article

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Biomaterials Porosity Determined by Fractal Dimensions Succolarity and Lacunarity on Micro-CT Images

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Abstract

Porous structures are becoming more and more important in biology and material science because it helps in reducing the density of the grafted material. For biomaterials, porosity also increases the accessibility of cells and vessels inside the grafted area. However, descriptors of porosity are scanty. We have used a series of biomaterials with different types of porosity (created by various porogens: fibers, beads...). Blocks were studied by microcomputed tomography for the measurement of 3D porosity. 2D sections were re-sliced to analyze the microarchitecture of the pores and were transferred to image analysis programs: star volumes, interconnectivity index, Minkowski-Bouligand and Kolmogorov fractal dimensions were determined. Lacunarity and succolarity, two recently described fractal dimensions, were also computed in special software written in MatLab. These parameters provided a precise description of porosity and pores' characteristics. Non-linear relationships were found between several descriptors e.g. succolarity and star volume of the material. A linear correlation was found between lacunarity and succolarity. These techniques appear suitable in the study of biomaterials usable as bone substitutes.

Keywords: Porosity; Connectivity; Fractal dimension; Lacunarity; Succolarity

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