GRIBOI

GROUPE DE RECHERCHE INTERDISCIPLINAIRE SUR LES BIOMATERIAUX OSTEOARTICULAIRES INJECTABLES

11th Interdisciplinary Research Conference on Biomaterials

Coquelles (Calais) France

8th - 9th March 2001

Université du Littoral
Côte d'Opale

RÉGION NORD PAS DE CALAIS

Fédération des Biomatériaux Nord/Pas-de-Calais

Thursday, March 8th

09.00 am : Registration

09.30 am : Welcome address

Session I : Physicochemical characterization of biomaterials

10.00 am : **Invited lecture : AP Legrand, B Bresson**
Laboratoire de Physique Quantique. ESPCI. Paris, France.
Bone and Bone Substitutes NMR Characterisation

10.45 am : EF Brès[1] and JL Hutchinson[2]
[1] ESA CNRS 8008, Université de Sciences et Technologies de Lille, France. [2]Department of Materials, University of Oxford, UK
Observation of the surface structure of biological calcium phosphate apatite crystals from human tooth enamel

11.00 am : EI Suvorova[1] and PA Buffat[2]
[1] Institute of Crystallography RAS, Moscow, Russia. [2] Centre Interdépartemental de Microscopie Electronique, EPFL, Lausanne, Suisse
Model of the Ca-loss mechanism in bones under microgravity and earth conditions

11.15 am : Coffee Break

11.45 am : SM Belkoff[1], H Deramond[2], LE Jasper[1] and JM Mathis[3]
[1]The University of Maryland, Baltimore, USA. [2]University Hospital, Amiens, France.
[3] Lewis-Gale Clinic, Salem, USA
Biomechanical evaluation of a hydroxyapatite cement for use with vertebroplasty

12.00 am : F Lefebvre, JJ Fabre, D Ahite and P Campistron
Institut d'Electronique et de Microélectronique du Nord, DOAE, Université de Valenciennes et du Hainaut Cambrésis, France
Development of a new ultrasonic technique for bone and biomaterials characterization

12.15 am : E Cau, G Penel and G Leroy
LBM Micro Raman, Faculté d'Odontologie, Université de Lille 2, France
Physicochemical investigations of natural and synthetic calcium carbonate

12.30 am : Lunch

Session II : Orthopaedic tissue engineering

02.00 pm : **Invited lecture : JT Triffitt, ROC Oreffo and AS Virdi**
Bone Research Laboratory, Nuffield Department of Orthopaedic Surgery, University of Oxford, UK
In vivo transplantation of genetically-marked human bone marrow osteoprogenitors and their localisation in skeletal sites

02.45 pm : K. Anselme, O. Broux, B. Noël, AF. Dudermel[1], F. Bianchi[1],
B. Bouxin[1], P. Hardouin and J. Jeanfils
I.R.2.B., Université du Littoral Cote d'Opale, Berck/mer, Boulogne/mer. [1] Institut Calot, Groupe Hopale, Berck /mer, France.
In vitro control of human bone marrow stromal cells for bone tissue engineering

03.00 pm : O Broux, C Chauveau, JC Devedjian , B Noël, C Delecourt[1],
K Anselme, MC Blary, B Flautre, D Darriet[1] , P Rigaux[1], F Danze[1],
P Hardouin and J Jeanfils
I.R.2.B., Université du Littoral Cote d'Opale, Berck/mer, Boulogne/mer. [1]Etablissement Héliomarin, Groupe Hopale, Berck/mer, France
RT-PCR analysis of human heterotopic ossification

03.15 pm : Coffee Break

03.30 pm : **Invited lecture : JA Hubbell**
Institute for Biomedical Engineering, University of Zürich, Switzerland
Biomimetic and in situ curing materials in bone regeneration and repair

04.15 pm : K Anselme, M Rouahi, B Noël, T Grard, O Gallet[1], JM Imhoff[1],
M Descamps[2], F Bianchi[3], AF Dudermel[3], B Bouxin[3],
P Hardouin and J Jeanfils
I.R.2.B., Université du Littoral Cote d'Opale, Berck/mer, Boulogne/mer. [1] ERRMEC, Université de Cergy- Pontoise. [2] LAMAC, CRITT Céramiques Fines, Maubeuge. [3] Institut Calot, Groupe Hopale, Berck/mer, France
Improving of hybrid materials thanks to autologous adhesion proteins

04.30 pm : GRIBOI General Council

05.00 pm : Departure to Boulogne sur mer

05.30 pm : Social event in Boulogne

07.00 pm : Reception in Boulogne City Hall by the Lordmayor

08.30 pm : Conference Dinner

Friday, March 9th

08.45 am : **Invited lecture : JM Planeix, MW Hosseini, EF Brès[1], J Czernuska**
Université de Strasbourg, [1] Université de Sciences et Technologies de Lille, France
A molecular tectonics/crystals engineering approach for building organic/inorganic composites. Potential applications to biomaterials

Session III : Calcium phosphate cements

09.30 am : A Brouchet[1], S Gonçalves[2,3], JL Lacout[2], G Viguier[1] and B Delisle[1]
[1] Service d'Anatomie et Cytologie Pathologique, CHU Rangueil, Toulouse. [2] CIRIMAT ENSCT-INPT, Toulouse. [3] TEKNIMED, Vic en Bigorre, France
Radio-histological study of two phosphocalcium injectable

09.45 am : O Gauthier[1,2], R Mueller[3], D Von Stechow[3], JM Bouler[1], G Daculsi[1] and E Aguado[1,2]
[1] Faculté de Chirurgie Dentaire, Nantes. [2] Ecole NationaleVétérinaire de Nantes. [3] Harvard Medical School, Boston, MA, USA
Bone substitution with injectable calcium phosphate biomaterial : in vivo three-dimensional micro-tomographic study

10.00 am : JX Lu[1], J. Dejou[1], JP Proust[1], P Hardouin[2], B Flautre[2], M Descamp[3], J Lemaître[4] and P Van Landuyt[4]
[1] Faculté d'Odontologie, Marseille. [2] IR2B, ULCO, Berck /mer,
[3] LAMAC, CRITT Céramiques Fines, Maubeuge. [4] Ecole Fédérale Polytechnique de Lausanne, Switzerland
The mechanism of biodegration of calcium phosphate biomaterials

10.15 am : B Flautre[1], P Van landuyt[2], C Maynou[3], J Lemaître[2] and P Hardouin[1]
[1] IR2B, ULCO, Berck/mer. [2]Ecole Fédérale Polytechniqùe de Lausanne, Switzerland,
[3] CHU B Lille, France
β-TCP granules and cement matrix interaction on the biological properties of brushite cements

10.30 am : K Dai and X Shang
Ninth People's Hospital, Shanghai Second Medical University, Shanghai, PR China
An experimental study of β-TCP impregnated bone cement and its bone –augmentation effect

10.45 am : Coffee break

11.15 am : Poster session

12.30 am : Lunch

Session IV : Miscellaneous and Clinical aspects

02.00 pm : T Franz[1,2], C Fankhauser[2], B Gasser[2] and PF Heini[1]
[1] University of Bern, Switzerland, [2] Dr HC Robert Mathys Foundation, Bettlach, Switzerland
PMMA bone cement augmentation - A possibility to increase the fracture load of the osteoporotic proximal femur ?

02.15 pm : C Wang, S Liu, S Wang, Q Zhao, J Bei, Q Cai and M Fan
Institute of Basic Medical Sciences of Beijing, China
Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human trachea cancelled

02.30 pm : Q Gu and Y Yan
Shanghai Qisheng Institute of Biomaterial and Technology, China
Viscosupplementation : a new concept for treatment of osteo-arthrosis in human with sodium hyaluronate

02.45 pm : H El Briak, D Durand, J Nurit, S Munier, B Pauvert and P Boudeville
Faculté de Pharmacie, Montpellier, France
Study of a hydraulic dicalcium phosphate dihydrate/calcium oxide-based cement for dental applications

03.00 pm : J Lu
Presentation of next meeting in Shanghai.

03.30 pm : Concluding Remarks

03.45 pm : Coffee break and departure

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SESSION I

PHYSICOCHEMICAL CHARACTERIZATION OF BIOMATERIALS

Bone and Bone Substitutes NMR Characterisation

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Keywords: ^{31}P & ^{1}H Solid State NMR, calcium phosphate ceramics, osteoformation

Abstract. High resolution solid state NMR spectroscopy appears as a powerful method for a better understanding of bone structure and bone implant. In particular it is efficient to estimate osteoformation via bioceramics bone colonisation. ^{31}P, ^{1}H NMR are generally used to the analysis of different type of bioceramics, to follow the transformation of calcium phosphate ceramics versus implantation time, to examine the influence of a protein....

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Observation of the surface structure of biological calcium phosphate apatite crystals from human tooth enamel

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Abstract

A surface structure study of human tooth enamel crystals has been carried out by high resolution electron microscopy (HREM). Although the difference of morphology in the crystals observed indicates that the growth control by matrix proteins takes only place on the {01-10} surfaces of the crystals. The crystal described here is oriented along the [11-20] direction and the following surfaces have been analysed: (1-100), (01-11), and (0001). A subsurface reconstruction is observed just below the surface of the crystal not bonded to the matrix and lying above the supporting film. Observation of the matrix surrounding the crystal shows the existence of poorly crystalline phases with a structure close to that of hydroxyapatite (OHAP). Finally, a comparison of the images of the surface with computer simulated images calculated for several models of the OHAP surface structure shows that the surface itself is stoichiometric and contains both calcium and phosphate groups. The knowledge of the binding sites of proteins on biomineral crystals such as the ones found in human tooth enamel is of prime importance for the understanding of their growth process as well as the interactions with apatite crystals surfaces. In this study, the first structure determination of the surface of human tooth enamel crystals is presented.

Model of the Ca-loss mechanism in bones under microgravity and earth conditions.

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Precipitation of Ca phosphates from aqueous solutions under conditions close to the physiological ones can serve as a simplified model for mineral phase formation (or destruction) in bone. It considers the physico-chemical processes in a system containing only calcium and phosphates ions in different concentrations. Supersaturation is the driving force for calcium phosphate formation. The rate of mixing of the stock solutions and the duration of crystallisation determine its value and distribution in the reaction chamber.

Examination by X-ray diffraction, scanning and transmission electron microscopy of hydroxyapatite and octacalcium phosphate materials precipitated under different conditions on earth and in space showed differences in phase composition, morphology, sizes and structure of crystals.

The lowest value of supersaturation can be reached exclusively in space where only diffusion is supposed to provide the mass transport. On earth, a very low speed of stock solution mixing is required to get the smallest supersaturation values. A higher speed of mixing increases the supersaturation what results in promotion of the nucleation rate but not in crystal growth. The size of the HAP crystals grown in space is 1.5 order of magnitude larger than the ones obtained on earth with similar conditions but with gravity in addition. HAP crystals of nanoscale can be synthesised only with a very high rate of mixing of the stock solutions and short duration of crystallisation (less 1 minute).

Single phase hydroxyapatite precipitation is obtained only if a homogeneous distribution of the supersaturation exists in the crystallisation chamber, for instance under gravity convection for a crystallisation time long enough or by controlled forced stirring. Heterogeneous distribution of the supersaturation happens in the case of either mass transport under diffusion control only (space), very small rate of mixing of the stock solutions or the high rate of mixing and together with short duration of crystallisation. This leads in all cases to the formation of multiphase precipitation (OCP + HAP) and polyphased crystals.

The larger sizes of crystals and the formation of two phases in space can break the dynamical equilibrium on Ca exchange between a solid (bone) and a liquid. This can lead to a loss of Ca by bones. Formation of mixed crystals on earth under bad mixing conditions, for instance during a long immobilisation of the body, can also disturb the equilibrium and result in pores.

BIOMECHANICAL EVALUATION OF A HYDROXYAPATITE CEMENT FOR USE WITH VERTEBROPLASTY

Belkoff, S.M., Deramond, H.*; Jasper, L.E. and Mathis, J.M.**

*The University of Maryland, Baltimore, USA; *University Hospital, Amiens, France; **Lewis-Gale Clinic, Salem, USA*

Purpose: To determine the strength and stiffness of osteoporotic vertebral bodies subjected to compression fractures and stabilized via bipedicular injections of: Simplex P (SP) (Stryker-Howmedica-Osteonics, Rutherford, NJ); Simplex P formulated consistent with the practice of vertebroplasty (F2), i.e. 30% $BaSO_4$ content by weight and a monomer-to-powder ratio of 0.71mL/g.; or BoneSource (BS) a hydroxyapatite cranial defect filler (Stryker-Howmedica-Osteonics, Rutherford, NJ).

Methods: Vertebral bodies (T8-T10 and L2-L4) from each of 10 fresh spines were harvested from female cadavers (81 ± 12 years), screened for bone density (t-score, –3.8 ± 1.1, bone mineral density, 0.75 ± 0.15 g/cm^2), disarticulated, and compressed to determine initial strength and stiffness[1]. The fractured vertebral bodies were stabilized via bipedicular injections of 4 cc (thoracic) or 6 cc (lumbar) and then recrushed. We checked for an effect of treatment (SP, F2, BS) and condition (initial *versus* after treatment) on VB stiffness and strength using a repeated measures ANOVA. Tukey's post hoc comparison test was conducted to determine if differences were significant ($P \leq 0.05$)

Results: Vertebral bodies repaired with Simplex P resulted in significantly greater strength relative to their prefracture states, those repaired with BoneSource resulted in the restoration of initial strength for both the thoracic and lumbar level, and those repaired with F2 resulted in significantly greater strength in the thoracic region and restoration of strength in the thoracic region and restoration of strength in the lumbar region. All treatments were significantly less stiff compared with initial values.

Table 1. Thoracic (T8-T10) VB Results

Parameter	SP Group	F2 Group	BS Group
Initial strength (N)	2522 ± 347	2710 ± 330	2336 ± 330
Treatment strength (N)	4058 ± 347	4146 ± 330	2476 ± 330
Initial stiffness (N/mm)	1559 ± 102	1783 ± 97	1298 ± 97
Treatment stiffness (N/mm)	1097 ± 102	1224 ± 97	797 ± 97
Initial failure deformation (mm)	2.1 ± 0.3	1.8 ± 0.3	2.2 ± 0.3
Treatment failure deformation (mm)	4.2 ± 0.3	4.0 ± 0.3	4.3 ± 0.3

Table 2. Lumbar (L2-L4) VB Results

Parameter	SP Group	F2 Group	BS Group
Initial strength (N)	2813 ± 364	2696 ± 364	2630 ± 364
Treatment strength (N)	4208 ± 364	3134 ± 364	2450 ± 364
Initial stiffness (N/mm)	1842 ± 74	1794 ± 74	1834 ± 74
Treatment stiffness (N/mm)	1371 ± 74	1301 ± 74	1301 ± 74
Initial failure deformation (mm)	1.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.3
Treatment failure deformation (mm)	4.0 ± 0.3	4.4 ± 0.3	3.8 ± 0.3

Conclusions: All three materials tested restored or increased vertebral body strength, but none restored stiffness. Both new materials show promise for use in percutaneous vertebroplasty, but they need clinical evaluation.

References: 1. Belkoff SM, Mathis JM, Erbe EM, Fenton DC. Biomechanical evaluation of a new bone cement for use in vertebroplasty. *Spine* 2000;25:1061-1064.

Development of a new ultrasonic technique for Bone and Biomaterials characterization.

F. LEFEBVRE, J.J. FABRE, D. AHITE, P. CAMPISTRON

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Ultrasonic techniques of bone caracterization have been presented in many reports over the last years. They have been seen as a consistent method which can evaluate bone state and pathology (osteoporosis, for example). Classical techniques based on bone imaging allow visual examination or provide quantitative parameters like Bone Mineral Density which is a mass per unit of surface. Unfortunately, these techniques are generaly heavy and expensive.

This paper presents a new method for bone caracterization using Lamb's wave which is able to evaluate mechanical properties like Young Modulus E and cortical bone thickness. The original aspect of the method is in the use of low frequency transducers to measure ultrasonic velocity. Due to the ponctual contact between the transducer and the material under examination, no coupling medium is needed. The method is thus easy to use with low cost apparatus (fig. 1). Three main points are discussed in this paper.

Firstly, we present briefly Lamb's wave propagation througth different geometrical model : plane model, cylindrical model and pipe. The two last models are performed to simulate long bone shape. Secondly, the correlations between propagation velocity of Lamb's wave and bone thickness are investigated on bovine bone samples. An artificial decalcification are done on bone samples in order to get bone density evaluation from ultrasonic data. Finally, Young Modulus and thickness estimations are presented and compared with the litterature values.

All these results show the ability of this technique for non destructive evaluation of mechanichal and geometrical properties of long bones and let us envisage in vivo measurement of those parameters. Moreover, in the case of biomaterial implants, our method can be used to follow up the bone regeneration or the implant resorption if it occurs. Mechanical properties of the bone-implant association can also be evaluated.

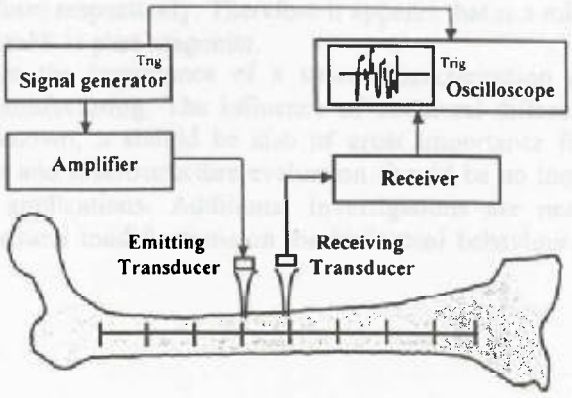

Fig. 1 - Experimental set-up.

PHYSICOCHEMICAL INVESTIGATIONS OF NATURAL AND SYNTHETIC CALCIUM CARBONATE

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Today the interest of bone substitute biomaterials is unanimously recognised. The biocompatibility of such materials must be excellent and they should be gradually resorbed and replaced by new bone formation. Calcium phosphates and calcium carbonates are widely used for orthopaedic and maxillo facial surgery. But the interactions between the material and the host bone are not yet perfectly understood. The interpretation of the results are difficult and sometimes discrepancies appears. It appears that these problems could have been encountered because of insufficient characterisation of the basic component used in the synthesis of such biomaterials. Due to the fact that their composition was not strictly identical a valid comparison between the final biomaterials was in many cases impossible. Because of the lack of suitable analytical techniques for their detection, the low concentration components were long considered negligible. The calcium carbonate ($CaCO_3$) is interesting in bone filling or regeneration procedures because of its resorbability. Different sources like coral or different sea shells are used to produce $CaCO_3$. The aim of this work is to compare differents $CaCO_3$ biomatérial and their basic source before manufacturing with the help of the microRaman spectroscopy. This technique is now efficient to obtain a strict characterisation, including low concentration components.

Bionacre® (Bionacre, France) Biocoral® (Inoteb, France) are analysed and compared to natural compound, *Tridacna gidas* shell, oyster shell, eggshell and, synthetic calcite and aragonite. All spectra were obtained from an OMAR 89 from DILOR (Lille, France), equipped with a microscope and laser excitation. The overall spectral resolution is 2 cm^{-1}.

The spectral analysis confirm the $CaCO_3$ composition of all the samples. The main bands of carbonate internal modes are observed around 1084-86 (ν_1 mode) and 704-12 cm^{-1} (ν_4 mode). In the lattice modes region some differences appears between samples. The frequency of lattice mode bands depends on the crystal structure. On Biocoral®, *Tridacna gidas* shell and, synthetic aragonite two bands at 206 and 153 cm^{-1} are observed. The eggshell, oyster shell and synthetic calcite samples exhibit bands at 281 and 155 cm^{-1}. Three bands are present at 280, 206 and 153 cm^{-1} on the Bionacre® sample. These differences are due to the structural changes between those calcium carbonates. The 206 and the 280 cm^{-1} bands are due to aragonite and calcite form respectively. Therefore it appears that is a mixture of aragonite and calcite whereas, Biocoral® is pure aragonite.

These results illustrate the importance of a strict characterisation of biomaterial at the different stages of manufacturing. The influence of structural differences between apatitic biomaterials is well known, it should be also of great importance for calcium carbonate. Chemical composition and macrostructure evaluation should be no longer sufficient to valid medical or surgical applications. Additional investigations are needed to evaluate the influence of such structural modifications on the biological behaviour of calcium carbonate bone substitutes.

SESSION II

ORTHOPAEDIC TISSUE ENGINEERING

In vivo transplantation of genetically-marked human bone marrow osteoprogenitors and their localisation in skeletal sites.

James T. Triffitt, Richard O.C.Oreffo and Amarjit S. Virdi

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University of Oxford, Nuffield Orthopaedic Centre, Oxford, OX3 7LD, UK.

The use of osteogenic stem cells or osteoprogenitors to reconstruct skeletal tissues is a popular area of research investigation with high potential for successful use of tissue engineering principles. There have been many investigations on the optimal procedures for progenitor cell amplification and differentiation in *in vitro* culture, but relatively little work on the *in vivo* fate of such cells after transplantation. The main aim of the present work is to determine cell fate after in vivo implantation. For this purpose we have selected osteoprogenitors from human bone marrow specimens by an immunological method using a monoclonal antibody, HOP-26, which was previously generated in our laboratory. This antibody has high reactivity with a cell surface antigen present on human osteoprogenitors in bone marrow fibroblast populations. The selected cells were cultured in serum-containing media which was supplemented with ascorbate-2-phosphate and dexamethasone to promote differentiation of the osteoblast phenotype. This differentiation was confirmed by expression of osteocalcin protein determined immunohistochemically, and Type I collagen and osteocalcin mRNA expressions determined by RT-PCR analysis. To be able to identify the selected progenitor cells and their progeny after in vivo implantation, the selected cells were genetically-labelled using a murine leukaemia virus (MuLV) encoding a reporter gene (*lacZ*) with a selective marker gene (neo^r) using a triple transient transfection protocol. Stably transfected cells were implanted in CB17 scid/scid mice by local subcutaneous injection over the calvariae. Localization of the genetically-marked cells, which expressed the reporter gene, within the calvarial tissues was detected by beta-galactosidase histochemistry and immunocytochemistry using an antibody directed against this bacterial enzyme. Cells derived from the genetically-marked cells were found to be located within the periosteal layers in the calvariae and in close association with the osteoblast layer, covering mineralized bone surfaces and within bone osteoid at 5 and 7 days after injection. Further work is required to determine the long term survival and activity of the injected progenitor cells. However, this current study demonstrates the successful selection, expansion and retroviral-marking of human osteoprogenitors and their migration and localization within calvariae of SCID mice following *in vivo* implantation. These basic studies demonstrate the migration of these marrow derived cells to a skeletal site. This work supports the possibilities for assessing the successful uses of human osteoprogenitors in therapy of bone deficiency disease. It further suggests the potential for development of gene therapy procedures in these conditions.

IN VITRO CONTROL OF HUMAN BONE MARROW STROMAL CELLS FOR BONE TISSUE ENGINEERING

K. Anselme, O. Broux, B. Noël, A-F. Dudermel*, F. Bianchi*, B. Bouxin*, P. Hardouin, J. Jeanfils

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** Institut Calot, Groupe Hopale, Berck sur mer, France.*

MSC = marrow stromal cells

Bone regeneration is needed for the therapy of numerous clinical indications. The repair of large defects is a significant problem faced by orthopaedic surgeons. Many approaches to replace bone tissue have been described : autografts, allografts, or artificial materials used alone or in combination with bone grafts. More recently, in order to produce artificial bone, tissue engineering strategies have been developed. Four different cell-based tissue engineering approaches have been described for the regeneration of bone. These strategies have been based on the implantation of (1) unfractionated fresh bone marrow, (2) purified cultured expanded MSCs, (3) differentiated osteoblasts and chondrocytes or (4) cells that have been modified genetically to express bone morphogenetic protein (BMP). For several years, we develop in our laboratory strategies for bone tissue engineering based on points (1) and (2). The objective of this work is to test various serum for their capacity to favour in vitro proliferation and differentiation of human MSCs. Notably, new serum substitutes without animal- or human-derived proteins and specifically developed for tissue engineering strategies are compared to others serum substitutes and to fetal calf serum or autologous plasma.

Human bone marrow is harvested from iliac crests of patients undergoing surgery for head necrosis treatment. Whole human marrow is centrifugated using a previously described technique which allow to concentrate nucleated cells and to recover ~100 ml of autologous plasma. Cells are inoculated at 2 10^5 nucleated cells / cm^2 in αMEM +/- 10^{-8} M dexamethasone with various sera: (a) a selected lot of fetal calf serum 15% (Eurobio, France), (b) autologous plasma 15%, (c) a serum substitute prepared with animal- or human-derived proteins: Ultroser G® (Life Technologie), (d) a serum substitute prepared with plant-derived proteins: Prolifix S3 or S6® (Biomedia, France), and (e) AB human serum (Biomedia, France). In vitro phenotype of MSCs is systematically characterized using different techniques (i) counting of colony forming units-fibroblastic (CFU-Fs) which is related to osteoprogenitor cells content in bone marrow, (ii) determination of their alkaline phosphatase activity (ALP) by histochemical staining (iii) and RT-PCR analysis of the protein expression by confluent cell layers stimulated or not by $1,25(OH)_2$ vitamin D_3.

Serum substitutes appear to produce more reproducible results concerning the number of ALP positive CFU-Fs compared to autologous, human or fetal calf serum. The RT-PCR assay shows that the cells cultured in all sera express ALP, collagen type I, and osteonectin ARNm. Only cells treated with $1,25(OH)_2$ vitamin D_3 express osteocalcin ARNm. However, cells cultured in human autologous plasma show very variable results with a lower ALP activity than in the other media even undetectable for some samples. Dexamethasone has not any stimulative effect on in vitro hMSCs proliferation or differentiation.

The possibility to culture and to maintain in vitro the phenotype of hMSC in serum substitutes free of animal or human proteins we demonstrate in this study introduces an array of new perspectives in bone tissue engineering and in particular for the strategies using cultured expanded MSCs.

RT-PCR analysis of human heterotopic ossification

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[*] *The first two authors contributed equally to this work.*

Heterotopic ossification (HO) is a formation of trabecular bone in sites where it is not normally present. HO is a possible complication of head injury and can develop in different kinds of joints. It appears within 6 months following injury and is asymptomatic, although it may cause pain, decreased motion, or in severe case complete joint ankylosis requiring surgical intervention.

The factors that cause HO remain to be determined and a better understanding of this phenomenon may provide substantial insight into both pathologic and normal bone formations. Indeed, HO presents an opportunity to study osteogenic induction mechanisms and their regulatory pathways.

In recent years, advances in human genetic have led to the identification of many genes involved in different steps of osteoblast differentiation. With such genes at hands it becomes increasingly easier to analyse expression pattern of HO cells.

Considering the lack of information on molecular mechanisms involved in HO development, we perform a study developing gene analysis of human HO by RT-PCR.

We first try to optimise the cell fractionation from bone resection, preserving RNA integrity. Collagenase digestion (0.25% in PBS) was tested for various times ranging from 10 to 60 min. After 10 and 15 min of collagenase digestion, we were able to isolate intact RNA from bone by the guanidium isothiocyanate extraction protocol.

Following fractionation, preliminary gene expression pattern was performed by PCR on cDNA prepared from these RNA. For this study we focused on the expression of gene markers of different stages of stem cell differentiation.

Analysis of the first results allow us to conclude that we obtain valuable material in order to study the master genes and molecular pathway involved in the induction of osteogenesis. Development of this molecular analysis with an increased number of genes would be invaluable in designing better treatments and prevention of HO.

This work was supported, in part, by grants from the Société Francaise de Rhumatologie (SFR).

IMPROVING OF HYBRID MATERIALS THANKS TO AUTOLOGOUS ADHESION PROTEINS

K. Anselme, M. Rouahi, B. Noël, T. Grard, O. Gallet*, J-M. Imhoff*, M. Descamps**, F. Bianchi^, A-F. Dudermel^, B. Bouxin^, P. Hardouin, J. Jeanfils

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**ERRMEC, Université de Cergy-Pontoise, Cergy-Pontoise, France.*
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The association of bone marrow stromal stem cells and a porous calcium phosphate ceramic vehicle forms hybrid materials with osteoinductive capacity in vivo. Adhesion proteins like fibronectin (FN) increase the adhesion of bone marrow stromal stem cells on biomaterials in vitro. The objective of this work was to purify a fibronectin from human plasma and to test its efficiency for improving human marrow stromal stem cells (hMSCs) attachment, proliferation and differentiation after culture in a porous ceramic which is our usual vehicule for hybrid materials preparation.

We used porous hydroxyapatite-based ceramic disks of 2mm in height with pores 400-500µm in diameter and interconnections ~130 µm in diameter. Fibronectin was extracted from frozen plasmas by elutions on gelatin- and heparin-containing affinity columns. Firstly, we evaluated the adsorption capacity of our ceramics using increasing concentrations of FN and we measured by an ELISA assay the quantity of non adsorbed FN. Secondly, the optimum FN concentration for hMSCs attachment in 24-wells polystyrene plates was determined using increasing concentration of adsorbed FN. Thirdly, we adsorbed FN at 1µg/ml on disks of porous ceramic and we impregnated these disks in a cell suspension with 10^6 hMSCs / ml. After 1 hour of attachment, cells attached in the ceramic disks were counted using a colorimetric method. Finally, MSCs cell suspension at 10^5, 5 10^5, 10^6, 5 10^6, 10^7 cells /ml were used for impregnation of FN-coated and control ceramic disks to form hybrid materials which were cultured during one month at 37°C in 24-wells plates. After one month in culture, hybrid materials were treated with calcitriol 10^{-8} M during 2 days. Culture medium was then harvested for osteocalcin and type I procollagen synthesis evaluation and cell number in hybrid materials was evaluated using a colorimetric method.

From the first experiment, we determined that the ceramic saturation was obtained with 600 µg of FN/g de ceramic. Secondly, the optimum concentration for hMSCs attachment on hFN was at least 0.5 µg/ml. In the third experiment, we did not observe any difference in the hMSCs attachment on FN-coated ceramics and on control ceramics. From the 4th experiment, we observed an effect of adsorbed FN on proliferation and osteocalcin and collagen synthesis notably when the hybrid materials were prepared with cell suspension with 5 10^5 or 10^6cells/ml. No proliferation was obtained in hybrid materials inoculated with 10^5cells/ml. With higher concentration of cells, FN have no more any effect on hMSCs proliferation and differentiation.

We demonstrated the possibility to prepare autologous FN from human blood plasma but we failed to demonstrate its efficiency at 1µg/ml for human hMSCs attachement in a porous ceramic material. However, proliferation and differentiation of hMSCs in the ceramic material were increased in presence of human FN notably when hybrid materials were prepared with intermediate cell concentrations.

Others concentrations of FN need to be tested in further experiments but we can now consider the preparation of autologous FN for human autologous hybrid material preparation.

SESSION III
CALCIUM PHOSPHATE CEMENTS

Radio-histological study of two phosphocalcium injectable

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Introduction:

In orthopaedic surgery, the loss or the reinforcement of osseous substances often requires filling of the defective part. In order to make the surgical operations easier we sought to make an injectable form. Phosphocalcium ionic cement Cementek® [1] was made injectable by use of several types of additives. Two of them are presented herein : polyethylenglycol (PEG) [2] and silicone. The osteointegration of the two hydroxyapatite injectable cement was studied in vivo and presented there.

Materials and methods :

The experimental animal model used was a closed femoral cavities epiphysary suggested by Pasquier[3]. Seventeen White New Zealand rabbits (male from 4 to 5,2 kg), divided into several groups were studied. Each group corresponded to a time of given retreat (3, 6, 9, 12, 24 and 48 weeks). After animal sacrifice, specimens were fixed in formalin, were embedded in methylmetacrylate with no previous decalcification, then stained with solochrom. Severals parameters such as: cement size, cement location, bone-cement contact, vascularization, bone neogenesis, osteointegration and inflammatory reactions were studied with a semi-quantitative method. The silicone and PEG cements were treated with the same procedure.

Results :

On preliminary study, executed at 3 and 6 weeks, we have found, on microscopic examination, that there was an osteointegration of the cement. Indeed, the periphery of the two cements (silicone and PEG), was colonised by lately formed osseous spans. Between the spans, the tissue was richly vascularized. There was no fibrous reaction, no inflammatory reaction, and the bone-cement contact was unwell defined. Others results will be studied and presented later.

Conclusion :

The first microscopic results, on the osseous tissue response, obtained with this new PEG or silicone injectable cements are encouraging. Indeed, the 2 products seems to be perfectly biocompatible. No inflammatory reaction was observed and there was a good osteointegration, which was done quickly (lower than 6 weeks). The cortical location was essential to observe a good osseous neogenesis and a good osteointegration. These results should be confirmed on the subsequent analysis realised at 9, 12, 24 and 48 weeks.

References:

[1] : Lacout, J-L., Hatim, Z., Frèche, M. "Procédé de préparation d'un biomatériau à base d'hydroxyapatite, biomatériau obtenu et application chirurgicale ou dentaire", French patent 9803459, 1998.
[2] : Gonçalves, S., Rodriguez, F., Lacout, J-L. "Formulation d'un ciment phosphocalcique injectable", Actualité en Biomatériaux, volume VI, 2000, à paraître.
[3] : Pasquier, G., Flautre, B., Blary, M.C., Anselme, K., Hardouin, P. "Injectable percutaneous bone biomaterials. An experimental study in a rabbit model". *J. of Materials Sci: Materials in Med.*, 1996, 7, 683.

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Bone substitution with injectable calcium phosphate biomaterial: in vivo three-dimensional micro-tomographic study.

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Abstract

This study investigated for the first time the *in vivo* performance of a composite injectable calcium phosphate bone substitute (IBS) with micro-computed tomography (μCT). The injectable biomaterial was obtained by the association of a biphasic calcium phosphate (BCP) ceramic mineral phase and a 3% solution of a cellulosic polymer (hydroxy-propyl-methyl cellulose) in a 50/50 weight ratio. The BCP particles were 200 to 500 μm in diameter.

The injectable material was implanted for 6 weeks in 7x10 mm osseous cylindrical defects at the distal end of rabbit femurs. Qualitative and quantitative histological examinations were performed on both two-dimensional classical scanning electron microscopy and three-dimensional μCT. Quantitative results about new bone formation and BCP resorption assessed were studied and compared for statistical purposes with variance analysis.
Extensive newly-formed bone apposition was noted with both 2D and 3D techniques that confirmed the results obtained from several previous studies with IBS. Bone colonization occurred extensively during the 6-week implantation period. Micro-computed tomography provided a three-dimensional approach to describe the new bone architecture. It showed that newly-formed bone was in perfect continuity with the trabecular host bone structure and demonstrated the total interconnectivity of the restored newly-formed bone network that developed inside the femoral defects. Both imaging techniques showed not only the development of bone ingrowth inside the defects but also the BCP degradation during the implantation period.

This study provided the first 3D description of bone substitution associated with the implantation of calcium phosphate bone substitute and demonstrated the ability of our IBS to restore the initial bone trabecular structure 6 weeks after implantation in rabbits. The non-destructive μCT imaging technique allowed not only to have a precise qualitative description of bone ingrowth but also to perform quantitative bone morphometry measurements. It allowed comparative subsequent SEM analysis on the same samples since they were not destroyed during the preparation for the first μCT evaluation. The whole resorption-bone substitution process could be described with the non-destructive μCT technique. The inter-granular spaces in IBS allowed the development of a totally interconnected new bone architecture.

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The mechanism of biodegradation of calcium phosphate biomaterials

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PURPOSE: Two different hypothesises of the mechanism of the biodegradation of biomaterials are presented: a process dependent on interstitial liquids and a second based on a cellular process. This study was undertaken to understand the biodegradation mechanisms of calcium phosphate biomaterials with different fabrications.

MATERIALS AND METHODS: To test this hypothesis, porous hydroxylapatite (HA), beta tricalcium phosphate (β-TCP) ceramics and calcium phosphate bone cement (CPC) were implanted into cavities drilled in rabbit femoral and tibiae condyles. After killing the animals at 2, 4, 12 and 24 weeks of implantation, histological investigation and histomorphometry were performed on undecalcified samples (n = 6 or 8).

RESULTS AND DISCUTIONS: The rate of material degradation in cancellous bone was approximately 10%, 15%, 30%, 60% at 2, 4, 12 and 24 weeks respectively in the CPC; 1-5% at 12 weeks and 3-8% at 24 weeks in the HA; and 30-45% at 12 weeks and 50-75% at 24 weeks in the β-TCP. The biodegradation presented a diminution of material volume from the periphery to the center as well as a particle formation with the phagocytosis by numerous macrophages and giant cells in the CPC. In the β-TCP, there was a pheripheral and central diminution of material volume as well as an absence of particle formation and visible phagocytosis. In contrast, a minimal degradation of material in the HA was noted.

CONCLUSION: We consider that the process of biodegradation is directly influenced by the type of crystallisation of the phosphocalcic material. The sintered phosphocalcic bioceramics formed at a high temperature exhibit good crystallisation and are primarily degraded by a process dependent on interstitial liquids. However, the phosphocalcic bone cement is formed by physicochemical crystallisation and is primarily degraded through a cellular process.

β-TCP GRANULES AND CEMENT MATRIX INTERACTION ON THE BIOLOGICAL PROPERTIES OF BRUSHITE CEMENTS

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The resorbability and ability of Brushite hydraulic cement composition (BHCs) to promote new bone formation have been investigated in vivo on sheep (2) and rabbit (1) model with different results: at 16 weeks on a rabbit model a complete replacement of BHC by bone tissue has been demonstrated whereas a slower process with larger volumes in a sheep model has been observed .The presence of β-TCP granules upper than 300μm inside the hardened cement might have also resorbability to favour the induction phenomena.

The aim of the study was to investigate, in histology, the biological response of β-TCP granules in two cement formulations A and B

Material and methods

Two BHCs compositons : 1) BHC-A consists in a powder mixture of monocalcium phosphate monohydrate(MCPM) , β-tricalcium phosphate (β–TCP), calcium sulfate hemihydrate (CSH) and dihydrogen sodium pyrophosphate(NaDHPP) mixed with 0.85M H2SO4 aqueous solution and 2) BHC-B consists in a powder mixture of MCPM, β–TCP and NaDHPP mixed with 0.10M H2SO4 aqueous solution .Upon hardening, the injectable cements are converted into dicalcium phosphate dihydrate (DCPD).Cylinders bone defects (diam x length=5x10mm) were drilled in the distal epiphysis of rabbit femurs and filled with the injectable material. Twenty female New Zealand were operated on both sides by randomisation under general anaesthesia. Microscopic observations were done on 20μm- thick-stained sections .

Results

In contrast to BHC-A, BHC-B cements resorb faster and promote the formation of low-quality new bone, showing an important lack of mineralisation with a soft unmineralized osteoid tissue at the residual cement/new bone interface with a faster degradation of their granules with the addition of polymers. In BHC-A cements, β-TCP granules are less degraded but infiltrated with new bone tissue with a more efficiency of the stimulation of bone remodelling probably in relation to a more efficient stability at the bone implant interface.

References

Ikenaga M.,Hardouin P., Lemaitre J., Andrianjatovo H., Flautre B. J. Biomed.Mater.Res.40(1)139-144,98.

Flautre B., Delecourt C., Blary M.C., Van Landuyt P . Lemaitre J., Hardouin P. Bone 25(2)35S-39S,99.

An Experimental Study of β-TCP Impregnated Bone Cement and Its Bone-Augmentation Effect

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Tricalcium Phosphate (β-TCP) particles in diameter of 150-300 μm was impregnated with PMMA powder and then mixed with PMMA monomer. It is found that the solidification time of □-TCP impregnated bone cement was similar to the pure PMMA cement but its polymeric temperature was much lower than that of the pure PMMA cement.

25%, 50% and 75% compressive fracture were produced in ox vertebral bodies in an in vitro study. βTCP impregnated PMMA and pure PMMA cement was injected respectively into the vertebral bodies after fracture reduction. Compressive test of the injected vertebral showed that the compression strength has no significant difference between the two groups, but it was higher than the non-fractured control group.

In the in vivo study, β-TCP impregnated PMMA cement was injected into the fractured rabbit femoral condyle after reduction. Histological investigation was made 4, 12, 20 weeks after injection. Tetracyclin and Alzarin Complexon were injected respectively 14 days and 2 days before sacrifice. For fluorescent labelling no obvious foreign body reaction could be found in the surrounding tissue. The β-TCP particles were gradually absorbed, and a large amount of new bone ingrowth was found inside the bone cement and provided a well bone-cement interlocking.

The study showed: 1. Injection of β-TCP impregnated PMMA cement into a reduced compressive fracture of vertebrae could produce a bone augmentation effect without tissue reaction. 2. β-TCP particles could be absorbed and followed by new bone ingrowth. It is beneficial for maintaining long term effect of bone-augmentation and preventing migration of the cement mass.

SESSION IV
MISCELLANEOUS AND CLINICAL ASPECTS

PMMA Bone Cement Augmentation – A Possibility to Increase the Fracture Load of the Osteoporotic Proximal Femur?

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Introduction: Osteoporosis-related fractures of the proximal femur are an important public healthcare concern and a major source of mortality and morbidity among the elderly. Efforts to maintain or increase bone mass in the elderly are based on systemic drug therapy, but pharmacological trials have at best demonstrated increases of only a few percent in femoral neck BMD [1]. Prevention of hip fractures in the elderly could be achieved by increasing the strength of the proximal femur using an injectable bone cement (Polymethylmethacrylate, PMMA). The aim of the present study was (i) to investigate the feasibility of PMMA injection into the proximal femur, and (ii) to study the effect of cement augmentation on the *in vitro* failure load of the osteoporotic proximal femur under two different loading configurations.
Methods: Twenty matched pairs of osteoporotic human cadaveric femurs were selected from Caucasian donors (median age, 76.0 years). The specimens were assigned randomly to two different groups of ten matched pairs, each group to be mechanically tested in a separate loading case. Of each pair, one femur was chosen for augmentation, with the contralateral femur serving as a control. A biopsy needle was used to inject a low-viscous PMMA bone cement (Palacos™ LV-40 with Gentamicin, Essex Chemie AG, Luzern, Switzerland) into the proximal femur. The fracture tests were conducted in a Zwick 1475 universal material testing machine (Zwick GmbH, Ulm, Germany). The first group of ten matched pairs was tested to failure in a configuration based on Pauwels' model of single-leg stance [2]. The second group of femoral pairs was loaded to simulate forces from a fall on the hip [3, 4]. For both configurations, load-displacement curves were recorded, and the fracture load and the energy absorption were calculated. The Wilcoxon signed rank test was used to test for differences in fracture load and energy absorption between the reinforced femurs and the native controls.
Results: Low-viscous PMMA bone cement was easy to inject, and volumes of 28 ml to 41 ml (mean, 36 ml) could be applied. After injection, a temperature elevation (mean, +22.1 K) was noted in all specimens, with peak temperatures measured at the posterior surface of the femoral neck ranging from 39.2°C to 49.2°C. The fractures observed in the native control femurs corresponded to those frequently seen *in vivo*. For both testing configurations, the load at fracture ($p < 0.002$) and the energy absorption ($p < 0.002$) were significantly increased for the reinforced (PMMA augmented) femurs in comparison to the contralateral native controls.
Discussion: Two distinct loading configurations were used to simulate clinically relevant hip fractures *in vitro*. For both conditions, reinforcement with PMMA significantly increased the fracture load and the energy absorption of the osteoporotic femur as compared to the contralateral control femurs. These results provide evidence, that it is possible to strengthen the osteoporotic proximal femur against fracture by prophylactic bone cement augmentation (femoroplasty) using PMMA. However, the biologic side effects (risk of thermal necrosis due to the exothermic polymerization of PMMA) exclude its application *in vivo*. Thus, there is a need for further evaluation of injectable bone cements with lower polymerization temperature, or alternative injectable bone substitutes with mechanical properties similar to PMMA.
References: [1] Riggs BL, Melton LJ (1992) N Engl J Med 327: 620-7; [2] Pauwels F (1980) In: Biomechanics of the Locomotor Apparatus. (rev. translation of the German edition, 1965), Springer-Verlag, Berlin; [3] Courtney AC et al. (1994) Calcif Tissue Int 55: 53-8; [4] Courtney AC et al. (1995) J Bone Joint Surg 77-A: 387-95

Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human trachea

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In recent years, several approaches for tracheal replacement have been described. These include the use of autogenous tissue, allografts, prosthetic materials, or a combination of approaches. To date, a totally satisfactory approach has not been achieved. This study evaluates the feasibility of growing tissue-engineered cartilage in the shape of a human trachea using chondrocytes seeded onto a synthetic biodegradable polymer fashioned in the shape of a ten-year-old child's trachea. A polymer template was formed in the shape of a human trachea using a nonwoven mesh of polyglycolic acid-polylactic acid. Chondrocytes isolated from bovine articular cartilage, as described by Klagsbrun, were counted and concentrated in a cell suspension containing 5×10^7 chondrocytes/ml. Each polyglycolic acid-polylactic acid template was seeded with chondrocytes and then implanted into subcutaneous pockets on the dorsa of 10 athymic mice. The three-dimensional structure was well maintained 12 weeks after implantation.

Specimens harvested 12 weeks after implantation and subjected to gross morphologic analysis demonstrated new cartilage formation. Similarly, histologic examination using a standard hematoxylin and eosin stain demonstrated cartilage formation in specimens. Fuschian aldehyde-Alcian blue stains of tissue sections from specimens exhibited purple staining consistent with the presence of sulfated glycosaminoglycans such as chondroitin sulfate. Safranin O stain demonstrated the presence of collagen and suggested the presence of type □ collagen, specific for cartilage. This finding was confirmed using a monoclonal antibody raised against human collagen type □ that crossreacts with bovine collagen type □ .The overall geometry of the experimental specimens closely resembled the complex structure of the child's trachea.

These findings demonstrate that polyglycolic acid-polylactic acid constructs can be fabricated in a very intricate configuration and seeded with chondrocytes to grow tracheal replacements in the shape of a spiral, which would be useful to repair potentially fatal tracheal defects created by large circumferential resections. We believe that refinements in surgical technique, the addition of an epithelial lining to our engineered cartilage, and creation of rings to permit flexibility will allow for extensive tracheal resection, with functional replacement and improved survival.

Key words: trachea chondrocyte tissue-engineering

Viscosupplementation: A New Concept for Treatment of Osteo-arthrosis in Human with Sodium Hyaluronate

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Abstract: The paper reviewed the treatment of osteoarthrosis with sodium hyaluronate(SH) by means of intraarticular injection for viscosupplementation since 1996. During the period of five years, there have been 425 patients with traumatic arthritis and osteoarthrosis of the knees and 160 patients with painful shoulder treated with SH therapy. The results show that more than 80% of the patients were improved or much improved. The incidence of side effects is approximately 1.5%, but none has been attributable to any of the SH themselves. It was demonstrated that the new therapy is the most preferable method to achieve or maintain great efficiency for each patient receiving injection once weekly for 3-5 times.

POSTER SESSION

Effects of resinous monomers on human odontoblast cytodifferentiation *in vitro*

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Objective: The main hard tissues of teeth are composed of dentin and enamel, synthesized by the mesenchyme-derived odontoblasts and the epithelial-derived ameloblasts respectively. Odontoblasts are highly differentiated post-mitotic cells secreting the organic matrix of dentin. Pathological conditions such as carious lesions and dental injuries are often lethal to the odontoblasts, which are then replaced by other pulp cells. These cells are able to differentiate into odontoblasts and produce a reparative dentin. In a recent work, we have shown that this physiological event can be reproduced *in vitro* (About *et al.*, Exp Cell Res, 2000). The purpose of this study was to evaluate *in vitro* the effects of resinous monomers on the odontoblasts cytodifferentiation.

Materials and Methods: Pulpal cores from freshly extracted, unerupted, human third molars were cultured in vitro with β-glycerophosphate (2mM). This cell culture model was used to evaluate the effects of TEGDMA (10 μM), HEMA (10 μM), UDMA (1 μM) and Bis-GMA (1 μM) on the cytodifferentiation of pulp fibroblasts into odontoblastic cells. The pulp cells were incubated with the monomers for 4 weeks and the culture medium containing the monomers was changed every other day. The effect of the monomers on the cytodifferentiation was studied by evaluating the odontoblast specific gene expression.

Results: In the absence of monomers and after 2 weeks, mineralization nodule formation was observed. The immunohistochemical study revealed that, as odontoblasts, pulp cells contributing to the nodule formation synthesized type I collagen, osteonectin, and dentin sialoprotein. In addition, Fourier Transform Infrared Microspectroscopy (FTIR) analysis showed that the mineral and organic composition of the nodules were characteristic of dentin (About *et al.*, Exp Cell Res, 2000). When the monomers were added to the culture medium at non-toxic concentrations, the effects of HEMA and Bis-GMA were more noticeable than that of TEGDMA and UDMA on Collagen-1, Osteonectin and DSP expression. However, all monomers significantly decreased DSP expression and completely inhibited the mineralization nodule formation.

Conclusions: The resinous monomers differentially affect the odontoblast cytodifferentiation and the process of dentin synthesis in vitro. This reflects, at least in part, the direct pulp capping and the reparative dentin secretion in vivo. This culture system can be used as a model for the study of the cytocompatibility of dental restorative materials.

INJECTABLE CALCIUM PHOSPHATE BIOMATERIAL FOR IMMEDIATE BONE GRAFTING OF EXTRACTION SOCKETS WITH STANDARDISED BONE DEFECTS : IN VIVO STUDY IN DOGS.

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Bone loss is often important after tooth extraction and the association of pre-existing bone loss, alveolar ridge damages and resorption decreases the abilities of an efficient placement of dental implants or prosthetic restoration. In such a context, many different bone substitutes have been proposed to restore alveolar bone loss.
This experimental in vivo study evaluated the biological properties of an injectable bone substitute (IBS), associating a hydroxypropylmethylcellulose (HPMC) polymer vector (E4M® 3% w/w) with a Biphasic Calcium Phosphate mineral phase (BCP, with a 60/40 hydroxyapatite/ β tricalcium phosphate weight ratio) composed of granules with 40 to 80 µm in diameter. This IBS was used to fill premolars extraction sockets.

Twelve mandibular premolars were extracted bilaterally from 3 dogs (a total of 24 extraction sites). A standardised vestibular bone defect was created with a dental bur on all extraction sockets. Extraction sites from the right mandible were immediately grafted with the IBS whereas, on the left one, the sockets were left unfilled as control sites. Radiological follow up and tomographic imaging were performed during the implantation period and before the animals were killed two months after implantation. Then, all extraction sockets were individually prepared for histological evaluation in both light and scanning electron microscopy. The alveolar bone volume and alveolar right height were evaluated and compared in grafted and control sites.

Qualitative histological studies showed that the IBS was able to support the extensive apposition of well-mineralized newly formed lamellar bone over the entire socket surface and prevented alveolar ridge bone loss in treated extraction sites. Quantitative evaluation showed that the alveolar bone volume was preserved in grafted sites despite the experimental vestibular defect. Specially, the treated extraction sites presented a rounder alveolar shape with a better preservation of the alveolar ridge height compared to the ungrafted ones.
The injectable bone substitute, composed of a polymeric vector and biphasic calcium phosphate, was effective in enhancing the bone filling of extraction sockets with standardised bone defect. These results could be predictive for the biological behavior of such a material when used in pathological conditions.

Orthopaedic Tissue Engineering: Use of Polyglycolide Membrane to Guide Bone Regeneration in Femoral Metaphyseal Defects in Rabbits.

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Objective: To study the application of absorbable Self-reinforced polyglycolide (SR-PGA) membranes in guiding bone regeneration of experimental bone defects in rabbits femoral metaphysis.

Methods: SR-PGA membranes (0.15 mm thick 10x10 size) were used in this study to over distal femoral metaphyseal bone defects (3.5 mm diameter) in 30 New Zealand rabbits. The rabbits were divided into 4 groups: (1) defects grafted with autograft plug, (2) defects grafted with autugraft plug and covered with SR-PGA membranes, (3) non-grafted defects, and (4) non-grafted defects covered with SR-PGA membranes. The rabbits were killed after 6, 12 and 24 weeks postoperatively. Histology and microradiography were used to evaluate the specimens.

Results: Membranes gave rise to no adverse effects except for fluid accumulation in four cases. Membranes were associated with fibrous tissue proliferation that led to the formation of a *Neomembrane* (a hybrid of fibrous-tissue PGA material). This *neomembrane* has prevented the invasion of non-grafted defects by fibrous tissue from outside. Osseous tissue ultimately has filled these defects and healed. Healing of some of non-grafted defects (not covered by SR-PGA membrane) was compromised. Healing of grafted defects showed no significant difference whether membranes were used or not.

Conclusion: SR-PGA membrane is biocompatible with metaphyseal bone. It can be successfully used to to guide bone regeneration of experimental bone defects in rabbits metaphysis. This method of treatment may obviate the need to graft such defects.

THE EFFECT OF MONOMER ON MCF-7 BREAST CANCER CELL VIABILITY

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Purpose: The spine is the site most often affected by metastases of breast and lung cancer. Some physicians use percutaneous vertebroplasty (PVP) to structurally augment vertebral bodies weakened by metastatic tumors. Recently, a vertebra containing a tumor that previously had undergone PVP was recovered post mortum [1]. The tumor exhibited a necrotic zone around the injected polymethylmethacrylate (PMMA) cement, thus leading the investigators to hypothesize an antitumoral effect of the MMA monomer. The purpose of this study was to investigate the effect of MMA toxicity on breast cancer cells.

Methods: Immortalized MCF-7 breast cancer cells were plated with standard growth media and incubated in a 95% humid environment (5% $C0_2$; 37^0C). Upon 50% confluence, the cells were treated for one hour with no monomer or with .001 to 20 mg/mL (monomer/growth media). Trypan blue and clonogenic assays were conducted (x3) on each treatment group.

Results: Trypan blue: on average, 50% cell death occurred at MMA concentration of 10 mg/mL (Figure 1). Clonogenic assay: MMA concentration of ≥ 5 mg/mL reduced MCF-7 cell proliferation (Figure 2).

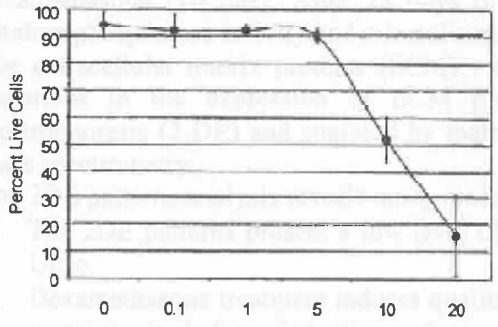

Figure 1

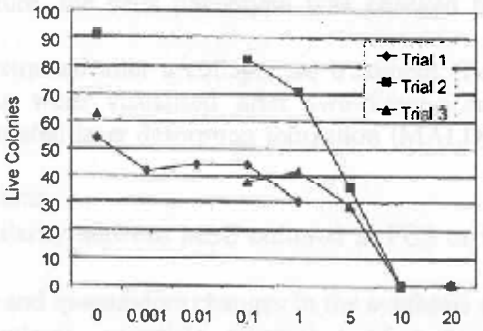

Figure 2

Discussion: Results of the present study indicate that MMA in concentrations ≥ 5 mg/mL is toxic to MCF-7 breast cancer cells. These results are similar to cytotoxicity results found for normal endothelial cells and leukocytes[2]. We found no reports regarding concentrations of monomer measured in vivo after PVP. Blood concentrations of MMA measured during total knee arthroplasty (TKA) were as high as 0.120 mg/mL, but rapidly decreased minutes after cementation[3]. Considering the volume of cement used in TKA is much greater than that used in PVP and that peak MMA/blood concentrations during TKA were 40 times less than MMA concentrations found to be cytotoxic to MCF-7 cells in the present study, MMA cytotoxicity may not be directly responsible for the tumor necrosis noted clinically after PVP [1].

References: 1. San Millan RD, et al., Pathology findings with acrylic implants. *Bone* 1999;25:85S-90S. **2.** Dahl OE, Garvik LJ, Lyberg T. Toxic effects of methylmethacrylate monomer on leukocytes and endothelial cells in vitro [published erratum appears in Acta Orthop Scand 1995 Aug;66(4):387]. *Acta Orthop Scand* 1994;65:147-153. **3.** Svartling N, Pfaffli P, Tarkkanen L. Blood levels and half-life of methylmethacrylate after tourniquet release during knee arthroplasty. *Arch Orthop Trauma Surg* 1986;105:36-39.

CHARACTERISATION OF OSTEOBLASTS DERIVED FROM BONE MARROW STROMAL STEM CELLS AND INFLUENCE OF CULTURE CONDITIONS ON ITS DIFFERENTIATION.

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In bone tissue engineering, new bone induction biomaterials could be established by association between porous hydroxyapatite (HA) and biological materials (proteins or cells) [1]. The preparation of this material requires the characterization of cells and biological active molecules during the bone differentiation.
In this study, we investigated the effects of culture conditions on the differentiation and we characterised differentiated bone marrow stromal stem cells.
Rabbit mesenchymal stromal stem cells (rabbit-MSC) were cultured in two differents serums substitutes (fetal calf serum 15% [FCS], Ultroser® 1% [Ultro]) with or without dexamethasone (10 nM). After 28 days of culture, the cells phenotype was checked by alkaline phosphatase activity and mineralization.
The extracellular matrix proteins (ECM) were extracted after a collagenase treatment. The variations in the expression of ECM proteins were visualised after two-dimensional electrophoresis (2-DE) and analysed by matrix assisted laser desorption ionization (MALDI) mass spectrometry.
The 2DE patterns analysis reveals many modifications.

- The 2DE patterns present a low level of similarity between MSC cultured in FCS or in Ultro.
- Dexamethasone treatment induces qualitative and quantitative changes in the synthesis of proteins, including induction of novel proteins, complete repression of proteins synthetised under basal conditions and significant increases in levels of other ones.
  Cells cultured in FCS and dexamethasone produced novel proteins which were localised in acidic area of pattern and in low moecular weight area whereas cells cultured in Ultro and dexamethasone didn't synthesize novel proteins in Ultro.
  The increase of rate protein synthesis induced by dexamethasone is higher for cells cultured in FCS than cells cultured in Ultro.

These results suggest that minor changes in the levels of specifc ECM proteins may have major effects on the cells phenotype. The cells characterisation and identification of biological active molecule (growth factors, proteins, ...) during the differentiation appear essential to produce new biomaterials based upon the association with biological material.

References.

1. Anselme, K.; Noël, B.; Flautre, B.; Blary, M.C.; Delecourt, C.; Descamps, M.; Hardouin, P. (1999) Association of porous hydroxyapatite and bone marrow cells for bone regeneration. *Bone* 25 (2): 51s-54s

Study of a Hydraulic Dicalcium Phosphate dihydrate/Calcium Oxide-based cement for dental applications

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Calcium hydroxide-based cements are commonly used in dentistry for endodontic treatments. The main advantage of calcium hydroxide is its biological activity : antibacterial and anti-inflammatory activities principally due to the high pH value of the surrounding environment following its dissolution. But calcium hydroxide also presents some drawbacks as pulp necrosis, low hardening and retraction by drying which does not allow tight fillings, consequently it is only used as temporary material for root canal filling.

We developed a calcium phosphate cement (CPC) made from monocalcium phosphate monohydrate (MCPM) and calcium oxide as solid phase and 1 M sodium phosphate buffer pH 7 as liquid phase [1]. This cement presents an antibacterial activity for calcium-to-phosphate ratios Ca/P $\geq$ 2. With these Ca/P, the compressive strength and the workability are correct with liquid-to-powder ratios L/P between 1–1.5, the expansion is 1–2 % during setting, but the initial setting time is short (3 – 4 min) and the injectability strongly decreases after 3.5 min. Consequently, this cement is suitable for pulp capping but not for root canal filling.

We present in this work a CPC made from dicalcium phosphate dihydrate (DCPD) and calcium oxide. Physico-mechanical properties as compressive strength, initial and final setting times, dimensional and thermal behavior, injectability, were investigated varying different parameters as L/P ratio, Ca/P ratio, phosphate buffer concentration and pH. The best compressive strength at one day, 12 MPa, was obtained for Ca/P = 1.67, L/P = 0.6 and a buffer concentration 0.75 M and pH 7. In these conditions : the initial and final setting times were respectively 15 and 30 min at 22 °C and 10 and 12 min at 37°C, the final setting time was 12 min at 37°C, the injectability was 100% up to 12 min, a low expansion around 0.7 % was observed and the inner temperature during setting did not exceed 50 °C. The increase in the Ca/P ratio, to improve the antibacterial effect of the cement, slightly decreased the compressive strength, the setting times T_i and T_f and the 100% injectability time but increased the expansion and the maximal temperature reached during setting : respectively 4.4 MPa, 8 min, 10 min, 7 min, 2.2 % and 55°C for the cement with Ca/P = 2.25 and L/P = 0.65). In the different experiments, the expansion was stabilized and the maximal temperature was reached at a time corresponding to the final setting time. The low time interval between T_i and T_f is very likely due to this heat evolution that accelerates the setting reaction.

After preparation, a root canal has generally a diameter below 1 mm and a depth of 15–21 mm, consequently the cement paste before setting has (i) to possess rheological properties allowing its penetration up to the apex but also into lateral canals and foramina and (ii) to be workable at least 15 min without loss of its final mechanical properties. Considering these constraints, the cement we developed from DCPD and CaO could serve for root canal filling as classical ZnO/eugenol-based pastes without or with gutta-percha points.

[1] S. Serraj, P. Michaïlesco, J. Margerit, B. Bernard and P. Boudeville. Study of a hydraulic calcium phosphate cement for dental applications ; 10th Interdisciplinary Research Conference on Injectable Biomaterials, Toulouse, 13-14 March 2000 ; submitted to *J. Mater. Sci. Mater Med.* June 2000.

Physicochemical mechanisms of injectable cement

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Introduction:

Phosphocalcium ionic cement Cementek® [1] was made injectable by the use of several types of additives. Two of them are presented herein : polyethylenglycol (PEG) and silicone. Using experimentals plan, two optimal formulations were defined [2]. After injection, the cements obtained lead to a final material made up of hydroxyapatite and preserve the main physicochemical characteristics of the product marketed. In this work, we present the various physicochemical mechanisms which modify the injectability, and the specific characteristics to these materials: mechanical properties, setting time, duration of injectability.

Materials and methods :

The influence of each additive on the rheological properties of the cement was measured. The injectability was correlated to the pressure required to move an 8 cm column of cement through a catheter of 3 mm internal diameter [3]. We characterised the products by infrared spectroscopy (Perkin-Elmer FTIR 1600), X-ray diffraction (CPS 120 INEL), mercury porosimetry (AutoPore III 9410) and scanning electron microscopy (LEO 435 VPS).

Discussion :

The various mechanisms which make injection possible are : interparticle sliding, sliding of cement on the walls of the catheter, modification of crystallite morphology , modification of cement rate evolution. It appears obvious that the lubricating properties of each additive improve the injectability of cements, however PEG and silicone had different effects. Thus, the modification of the size of the crystallites was observed for cements with PEG, while the presence of silicone modified evolution rate. The first histological results underline good osteointegration and biocompatibility of the various cements (the animal study will be presented in another communication of the congress).

This work was supported by a CEE grant (Craft).

References:

[1] : Lacout, J-L., Hatim, Z., Frèche, M. "Procédé de préparation d'un biomatériau à base d'hydroxyapatite, biomatériau obtenu et application chirurgicale ou dentaire", Brevet France 9803459, 1998.

[2] : Gonçalves, S., Rodriguez, F., Lacout, J-L. "Formulation d'un ciment phosphocalcique injectable", Actualité en Biomatériaux, volume VI, 2000, impress.

[3] : Leroux, L., Hatim, Z., Frèche, M., Lacout, J-L. "Effect of various adjuvants on the injectability of a calcium phosphate cement", *Bone*, 1999, 25 (n°2), 31S.

Glass-ionomer cements : fluoride and strontium release and uptake by dental enamel

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To prevent tooth decay during orthodontic treatments, fluoride treatments may be useful in hardening dental enamel. Glass-ionomer cements have found use as adhesive systems for brackets holding Ni-Ti wires. The organic resin fraction reacts with the inorganic filters and simultaneously with the apatite enclosed in the enamel while hardening. Following orthodontic treatment, the brackets are removed with minima damage to the enamel.

Release of fluoride ions from glass-ionomer cements is well documented. Strontium is also present in the filler portion to provide radio-opacity. We have studied fluoride and strontium concentrations found near enamel surfaces of freshly extracted molar after glass-ionomer treatment for up to 120 days.

Analysis by WDS (wavelength dispersive spectrometry) and by chemical analysis confirms that both fluoride and strontium ions are released by glass-ionomer cements and are effectively found fixed in the enamel. Electron microscopy shows the ions to penetrate the outermost 15 microns enamel. The kinetic studies by chemical analysis show that strontium enters the enamel apatite crystals faster than fluoride does. Acid etching of tooth enamel decreases the rate of ion fixation which can be related to a decrease in surface concentration of apatite crystals. No migration of aluminium or silicon was observed. The clinical significance of strontium uptake remains to be determined.

Substitutions in Fluorapatite and Clinical Applications.

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Abstract:

Fluorapatite, $Ca_{10}(PO_4)_6F_2$, is a phosphocalcic component present in several domains and particularly in biology. The human hard tissue contains a small part of fluorapatite. Its main component, fluoride, can be found in various natural sources: soils, waters, air, foods at different ratios. Fluoride is also used in two therapeutic forms (systemic or topical) for its beneficial action in caries prevention, and may be used against osteoporosis. Despite of the goods results of this method, the use of fluoridated water or complemented foods in a caries prevention context is more and more controversed, in regard to the risks of fluorosis. Fluorapatite belongs to the spatial group $P6_3/m$ (C_{6h}^2) and is constituted by 3 ions : F^-, Ca^{2+}, PO_4^{3-}. The fluorapatite structure and the presence of quantities of ionic bonds make fluorapatite a very pleasant host for lots of substituants, harmless or poisonous for the human organism. According the substitution site, we can describe 4 types of substitution. The F^- substitution, also called Type A substitution, is the main one, and the well-known. Only the Ca^{2+} substitution implies some changes in the crystal structure. However, all the substitutions types are well-known, which implies some unresolved questions.

«LitAr» – COLLAGEN-APATITE BIODEGRADABLE IMPLANT FOR ORTHOPEDY, OTORHINOLARYNGOLOGY, STOMATOLOGY.

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This implant (for prostheting the bone tissue defects) is a compositional substance on the base of collagen and hydroxysalts: calcium hydroxyphosphate and magnesium and aluminium hydroxyphosphate or hydroxysulphate. We considered the permission to use only calcium hydroxyphosphate or hydroxyapatite $[Ca_{10}(OH)_2(PO_4)_6]$ or simple apatite in medical practice. That's why we are going to present in the report the results of testing the material „LitAr", biological testing and clinical usage of implants on the base of hydroxyapatite only. Prosthetics of the bone tissue defects is the most efficient method when the biodegradable natural or artificial (synthetic) implantable materials are used. With the purpose of obtaining the highly efficient biodegradable implant " LitAr " the directed ionic diffusion (Ca^{2+},OH^-, PO_4^{3-}) in collagen was used. With this method we were able to achieve the high degree of structural integrability of the salt (hydroxyapatite) and collagen component. The availability of hydroxyapatite was supposed by X-ray phase analysis. The salt content in the implant was variable from 75% to 85%. The material behaviour was checked (in vitro and in vivo) by chemical analysis, radiology, scintigraphy, sonography and morphology. The results of the computed tomography investigation of the compound „LitAr" are shown: the salt distribution character in the course of all volume scanning the specimen is even (regularely). In this case all the density values in units H fluctuate in the space from 30 to 70 H. The X – ray density distribution histogram from which it follows that on the whole (60 %) of the material „LitAr" corresponds to the density is equel to the spase from 20 – 40 H. All the biological investigations were conducted on mongrel dogs by placing the implant into the trepanized hole of jaw, shin, costal bones and cranial bones. Successful biological testing the substance made possible the usage of the „LitAr" at the clinic of orthopedy, traumatology and urgent surgery of the Samara State University of Medicine and other clinics. From a great number of examples of the use of the „LitAr" we would like to give the most interesting aspects: 1)substituting the bone defect in the mastoidel bone (mastoidelplastic), postoperative forming the native bone in the defect zone under radiology control. Time of regeneration is equal to about 3 – 4 weeks ; 2) substituting the bone defect after chronic localized parodontitis (serious extent, the tooth was moved), after the access to the bone defect was provided through the trepanized hole made in the zone of the pulpal chamber; the defect is filled with the implant „LitAr" ; the zone was filling for 20 days. We were able to clearly see the bone tissue formation in the defect zone, and moving the tooth was finished; 3) another example of the clinic use of the „LitAr" for filling the defect (15x2x1.5cm) of the shin bone of patient A with the diagnosis "fibrous displosia", the pharmacokinetics curves showed biotransformation of the „LitAr" to the native bone for 3-4 months (the same time was for experimental animals). This year we have already begun using the compound „LitAr" in the form of a suspension ; it was performed by an injection method. The data of the measurement of the density by computed tomography in the standard parts correspondingly from 225 – 246 H (immidiatlly after injection „LitAr" to the defect zone) to 364 – 457 H (after 2.5 months of injection to defect zone) and 1080 – 1160 H (native bone in both cases). In the present paper we have generalized our experience in making, biological testing and clinical using the implant material „LitAr" of the new type and the latest generation, - the material which outperforms the biodegradable materials used before. This experience is without question positive, and therefore it is rather valuable.

Human osteoblast in contact with various bone cements *in vitro*

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PURPOSE: The aim of the study was to investigate changes in the number of osteoblasts and their viability as they are in contact with bone cements.

MATERIALS AND METHODS: Human primary culture osteoblasts were placed in contact with the extractive medium of cement polymethylmetacrylate (PMMA, CEMEX low viscosity radio-opaque, Tecres Italy), phosphocalcic cement A (CPC-A: β-TCP, MCPM, CSH, $Na_2H_2P_2O_7$ and H_2SO_4), and phosphocalcic cement B (CPC-B: β-TCP, MCPM, $Na_2H_2P_2O_7$ and H_2SO_4). After 48h in culture they were studied with an MTT viability assay as well as for cytotoxicity. The osteoblasts were cultured on the surfaces of the bone cements (PMMA, CPC-A and CPC-B). Cell proliferation was studied for cytocompatibility initially at 4 h, then at the seventh day, 14th, 21st and finally at the 31st day culture.

RESULTS AND DISCUTION: There was no difference in cell viability measured by means of MTT assay among osteoblasts cultured in control, PMMA and CPC-B during 48 hours of study, but CPC-A indicated a low cytotoxicity. The pH of the retrieved medium showed a minimal decrease in control and PMMA, a decrease in CPC-B, but an increase in CPC-A. A cement disaggregation in the culture medium with time was more pronounced in the CPC-A than in the CPC-B. We supposed that the cement crystallisation was weaker in the CPC-A than in the CPC-B. The cellular attachment on the cement surface after 4h of incubation was classed: CPC-B > Control > PMMA > CPC-A. The osteoblast proliferation increased with time in the control and PMMA, however, in the CPC-A and CPC-B, an increase was noted in the first 7 days followed by a decrease resulting in an absence of cells noted by the 21st day, secondary to the disaggretation.

CONCLUSION: *In vitro*, the two CPC presented an important disaggregation of cement, especially after 21 days of incubation, which influenced strongly the attachment and the cellular proliferation. In addition, the presence of an acid in the culture medium influenced the cellular proliferation of the osteoblasts.

Isolation and two-dimensional electrophoresis analysis of plasma membrane proteins from osteoblastic cells

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In bone tissue engineering, new bone induction biomaterials could be established by association between porous hydroxylapatite, titanium or glass-apatite and biological materials (proteins or cells) [1]. The material preparation requires the characterization of cells and biological active molecules during the bone adhesion and differentiation.

In this report, we have optimized a membrane plasma proteins purification protocol to study the effect of area topography on these proteins expression.

The expression of plasma membrane proteins from MG63 cells (osteosarcoma) was examined by cell disruption. Cells were fractioned using Dounce homogenisation followed by differential centrifugation[2,3]. The final supernatant contains plasma membrane proteins solubilized in Chaps [3-(-3-cholamidopropyl)dimehtylammonio-1-propane-sulfonate)].

The membrane fraction purity was examined and checked using enzyme markers assays such as lysosome, endoplasmic reticulum, mitochondria and plasma membranes.

The expression of plasma membrane proteins was characterized by two-dimensional electrophoresis (2-DE) analyses revealed by silver nitrate. The use of specific antibodies against major membrane proteins will allow us to study their expression in different conditions of area topography[4].

References.

1. Anselme, K.; Noël, B.; Flautre, B.; Blary, M.C.; Delecourt, C.; Descamps, M.; Hardouin, P. (1999) Association of porous hydroxyapatite and bone marrow cells for bone regeneration. *Bone* 25 (2): 51s-54s
2. Radisson, J.; Angrand, M.; Chavassieux, P. ; Roux, B. ; Azzar, G. (1996) Differential solubilization of osteoblastic alkaline phosphatase from human promary bone cell cultures. *Int. J. Biochem Cell. Biol.* 28(4): 421-430
3. Bourrat, C.; Radisson, J.; Chavassieux, P.; Azzar, G. ; Roux, B. ; Meunier, P.J. (2000) *Calcif. Tissue Int.* 66 :22-28
4. Grzesik, W.J. ; Robey, P.G. Bone matrix RGD glycoproteins : immunolocalization and interaction with human primary osteoblastic bone cells in vitro (1994) 9(4): 487-496